Recovery of small organics from natural sources using membrane technology.

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Recovery of Small Organics from Natural Sources using Membrane Technology

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Summary

The objective of this work was to investigate the use of membrane technology, and in particular nanofiltration, for the separation and purification of small bioactives from natural sources. Natural sources provide a virtually unlimited feedstock of complex compounds that are often very difficult to replicate synthetically. Separation methods are usually chemically/energy intensive that not only produce an expensive product but also one with a high environmental impact. Membrane technology provides an excellent alternative to traditional separation methods. Nanofiltration, is rapidly becoming a widely utilised separation method, with applications ranging from desalination pre-treatment to effluent purification. This thesis investigates the use of membrane technology for the purification of compounds from both fungal and potato feedstocks. Furthermore, a series of experiments were undertaken to investigate further the complex interfacial events that occur during nanofiltration.

This thesis characterised the effects of mass transfer and concentration polarisation in three commercially available frontal filtration systems. The work produced a new mass transfer correlation to account for the increased mass transfer effects observed with nanofiltration when compared to ultrafiltration. The hindrance factors associated with nanofiltration were experimentally derived and compared to theoretically obtained values. Good correlation was found which would allow researchers to confidently predict hindrance factors using the theoretically derived values. Experimental studies were undertaken to study salt rejection at the membrane's iso-electric point in order to investigate and increase understanding of the role of dielectric exclusion in nanofiltration. This study shows that screening of the dielectric effect is taking place at high salt concentrations.

On a laboratory scale, membrane technology was shown to be feasible for the purification and concentration of bioactives from a fungal (*Metarhizium anisopliae)* source. The results showed that not only did membrane technology provide a 'greener' separation method but also resulted in a more biologically active product with far greater commercial value.

Calystegines have already shown a number of interesting bioactive properties which have not yet been fully evaluated due to the small quantities extracted. The study focused on optimising the extraction process, prior to a series of laboratory scale feasibility studies and pilot scale membrane trials. The results demonstrated that membrane technology is suitable for the large scale purification of calystegines from waste potato peel.

Overall, this thesis has demonstrated the application membrane technology for the novel separation of bioactive compounds from natural sources. The studies undertaken have shown that nanofiltration is suitable for both laboratory based screening of compounds and large scale purification and recovery of small compounds. The findings will enable further laboratory scale research of membrane technologies as well as providing an alternative method for large scale biological separations.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed ..... (*Candidate*: Steffan Richard Williams)

Date 10 - June - 15

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed ..... (*Candidate*: Steffan Richard Williams)

Date 10 - June - 15

Signed: ..... (*Supervisor*: Dr Darren L. Oatley-Radcliffe)

Date: 15 June 2015

STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans **after expiry of a bar on access approved by the Swansea University.**

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List of Publications Arising from this Work

Published Papers

Oatley-Radcliffe, D.L., **S.R. Williams**, M.S. Barrow, P.M. Williams, (2014), Critical Appraisal of current nanofiltration modelling strategies for seawater desalination and further insights on dielectric exclusion, Desalination, 343, 154 - 161

Oatley-Radcliffe, D.L., **S.R. Williams,** T.J. Ainscough, C. Lee, D.J. Johnson, P.M. Williams, Experimental determination of the hydrodynamic forces within nanofiltration membranes and evaluation of the current theoretical descriptions, Separation and Purification Technology

Submitted Papers

Oatley-Radcliffe, D.L., **S.R. Williams**, C. Lee, P.M. Williams, Mass transfer characterisation of three commercial frontal filtration membrane systems, Desalination

Oatley-Radcliffe, D.L., **S.R. Williams,** C.P. Llenas, M. Brown, R. Nash, Generating a Value Stream from Potato Peel Waste: Composition and Calystegine Extraction Study, Food Chemistry

Conference Papers (* denotes presenter)

S.R.Williams*, D.L. Oatley, Investigation of charge properties of nanofiltration membranes at the membrane isoelectric point, Desalination using Membrane Technology, 7-10 April 2013 (Oral Presentation)

S.R.Williams*, D. L. Oatley, A. Abdrahman, T. Butt, R. Nash, Membrane Technology for the Improved Separation of Bioactive Compounds, Euromembrane Conference 2012, 23-27 September 2012 (Poster Presentation)

S.R.Williams*, D. L. Oatley, Membrane technology for the improved separation of bioactive compounds, IChemE Fluid Separations Special Interest Group, 'What's New in Fluid Separations 2012?', Basingstoke, 16 May 2012 (Oral Presentation)

S.R.Williams*, D. L. Oatley, Investigation of charge properties of nanofiltration membranes at the membrane isoelectric point, IChemE Fluid Separations Special Interest Group, 'What's New in Fluid Separations 2013?', London, 5 June 2013 (Oral Presentation)

S.R.Williams*, D. L. Oatley, Separation and Purification of Fungal Bioactives - A Membrane Approach, IChemE Fluid Separations Special Interest Group, 'What's New in Fluid Separations 2014?', Macclesfield, 20 June 2014 (Oral Presentation)

I. Garrido-Jurado*, **S.R.Williams**, A. Abdrahman, D. L. Oatley-Radcliffe, E. Quesada-Moraga, T.M. Butt, Non-target aquatic athropods testing of *Metarhizium* strains and their crude extracts produced by solvent extraction and nanofiltration technology, International Congress in Invertebrate Pathology and Microbial Control, 3-7 August 2014 (Oral Presentation)

1.0 Introduction

The separation, concentration and purification of chemical and biological mixtures continue to be a major area of interest at both pilot and industrial scales (Noble, 2005). The need to obtain high quality pure products is essential to many industries, ranging from oil and heavy chemicals to food, water and pharmaceuticals. The need for more efficient and effective separation techniques has become apparent in recent years, with greater emphasis placed on process cost reduction due to increasing economic pressures combined with more importance on developing sustainable processes to satisfy government environmental targets.

The work contained within this thesis attempts to further expand the applications of nanofiltration by using the technology for the separation and purification of bioactives from natural sources. The use of nanofiltration is ever expanding, however the uptake of the technology for biological applications is trailing behind chemical applications. Furthermore, the work undertaken attempts to provide increased understanding of the extremely complex transport and rejection mechanisms of nanofiltration. A sound theoretical understanding of the nanofiltration process is essential in order to promote widespread application of the technology. Theoretical descriptions are useful and inherently linked to any separation for the design, optimisation and scale-up of the resultant process.

1.1 Bio-technology and Bio-processing

Natural products are typically classified as organic materials derived from animal or plant extracts. Biotechnology has undergone phenomenal growth in recent years, capitalising on advancements in utilisation of biochemical synthesis routes for the manufacture of a wide range of products. Despite this growth in biotechnology sub areas such as bioprocess engineering have received much less funding, both in academia and industry, resulting in somewhat unfulfilled expectations (Ghosh, 2006). Biotechnology offers numerous advantages over purely chemical synthesis of both bulk and low-volume speciality products. A major advantage is the use of natural renewable feedstock, an important consideration in the long-term manufacture of products, for both economic and sustainability factors. Biochemical

processes are typically operated at ambient temperatures; hence have generally modest energy requirements when compared with conventional chemical processes (Weatherley, 1994). The adoption of biological sources, as for example the basis to a pharmaceutical, has the potential to simplify processes, reducing the heat-transfer, handling and storage requirements, and therefore reducing the overall number of processing steps. Other advantages of biological sources are the complexity and stereo specificity of the molecules produced, characteristics of particular importance in the manufacture of certain fungicides, pesticides, nutraceuticals and pharmaceuticals.

Despite the many positives which will be discussed in greater detail later, biotechnological processes do have an inherent number of fundamental separation challenges and difficulties that an engineer must overcome. The majority of bioreactor product streams are very dilute aqueous media. Regardless of the separation methods employed, the concentration of the solute/removal of the solvent is essential. Another inherent issue is the shear-sensitivity and complex rheological properties that biological mixtures and products display. Therefore, selection of appropriate and optimum separation methods are of paramount importance. The rheological, and interfacial behaviour of a biological system is likely to be time dependent, therefore, fast and efficient separations are essential. The problems of separating biological products are numerous and pose many challenges to the engineer when separating complex molecules from multi-component mixtures at low concentrations. The separations are often from environments that are physically awkward and that are prone to sensitive chemical and physical changes with respect to time. The products are also likely to be sensitive in nature, often requiring a controlled environment with respect to pH, temperature and concentration. A particular concern for products destined for say the food or pharmaceutical industry is product purity and sterility, for which strict specifications must be adhered to. Each of these factors has a profound influence on the selection of the separation process and upon the design of the separation process as a whole.

A major driving force in the growth of the biotechnology industry is the requirement for more complex and specific health care products, such as therapeutic proteins. All of these applications begin as bench scale feasibility studies even if the final product scale is only destined to be a specialist low-volume high-value product. The inherent bio-separations required are not necessarily easier and tend to be small scale processes. Conversely, the product discovered in the bench scale research and development may reside at the opposite end of the volume/value spectrum for biological products. Examples are the common antibiotic, penicillin (fungal derived) or the number one selling drug in the world, the statin Lipitor (fungal derived) which often involve elaborate separations, mainly to deal with the large process volumes but also to maintain sterility and control on such a large scale. However, irrespective of the final scale of product the need to select an appropriate separation process is essential in the feasibility of a new chemical entity (NCE).

Increasing environmental legislation and monitoring results in companies shifting towards leaner and cleaner processing, often predominantly to save money in tight economic climates with the added benefit of a cleaner process. The shift demands more in particular from the separation and recycling processes to help ensure cleaner environmental discharge, more efficient use of raw materials and energy to minimise the quantity of unwanted by-products and a reduction in un-reacted feed stocks and water use where possible. The waste water treatment industry, arguably the largest application of bioprocess technology on a mass basis worldwide (Aivasidis and Diamantis, 2005), are under increased pressure to meet higher standards and are therefore the leaders in the investigation and optimisation of bioseparation processes. This applies not only to municipal waste water treatment but also to processing plant waste water, where an efficient recycling process can signify a dramatic water volume usage reduction and hence a reduction in operating costs. The significance of effluent treatment is highlighted by Montgomery (2004) who states that 'the biological world does not synthesise materials that it cannot decompose, but with the introduction of organic chemical syntheses in the 19th century' led to products that could not be naturally

decomposed, hence leading to many of the pollution problems that exist today. This statements contains two significant points, one highlights the potential of using a product from a natural source eliminating polluting by-products, the other being the requirement of efficient separation methods for the treatment of existing waste products. Recent years has seen a change in the attitude of the wastewater industry. The increased stress on freshwater supplies due to a combination of consumer demand and climate change concerns has seen a significant increase in wastewater reuse and recycling (Tram et al., 2014).

Overall the exploitation of bio resources is growing year on year (Dolle, 2000); however, the quantity of natural products discovered and developed remains short of the true potential. Bio-processing sometimes referred to as downstream processing is a vital sub sector of biotechnology, essential for furthering the success in manufacturing bio-chemicals, biopharmaceuticals, foods, nutraceuticals, and agrochemicals. The application of efficient high throughput bio-processing separation and analysis techniques would allow for the natural product discovery rate to be accelerated. Downstream processing covers the application of a separation process for the recovery of biological processes, essentially any products that are produced through the use of any biochemical actions such as extractions and fermentations.

Bio-processing can be broadly categorised into two fairly distinct processes:

1. Reactive bio-processing
2. Extractive bio-processing

Reactive bio-processing covers the separation of product following a biological reaction, whereas extractive bio-processing involves almost exclusively bio-separations. However in both cases the products may not always be easily accessible and may require steps such as cell disruption to release intercellular products. Biological products can be classified by the sector of the products application as shown in Table 1.1. In reality the boundaries between intended applications are not as definitive and often product transfer is observed either as a pre-cursor or as an additive to a final product.

Table 1.1: Biological products based on application area (Adapted from Ghosh 2006)

Sector	Product examples
Industrial chemicals	Solvents, organic acids, industrial enzymes
Agrochemicals	Biofertilisers, biopesticides
Biopharmaceuticals	Antibiotics, hormones, monoclonal antibodies, plasma proteins, vaccines, hormones, cytokines, therapeutic nucleic acids
Food and food additives	Whey proteins, milk proteins, egg proteins, soy proteins, protein hydrolysates
Nutraceuticals	Vitamins, enzymes, coenzymes, cofactors, amino acids, purified whey proteins
Diagnostic products	Glucose oxidase, peroxidise, HCG
Commodity chemicals	Detergent enzymes, insecticides
Laboratory reagents	Bovine serum albumin, ovalbumin, lysozyme
Cosmetic products	Plant extracts, animal tissue extracts

The food industry has driven the development of bio-processing, probably due to the very large volumes of product and subsequent waste produced in the industry. The optimisation and maximal exploitation of every stream in the industry has led to the development of a new sector in recent years called 'nutraceuticals' (combination of nutrition and pharmaceutical, a food substance that provides health or medical benefit (Kalra, 2003; Aruoma, 2010)), a prime example being the production of whey protein from what used to be a waste product of the dairy industry. Nutraceuticals differ from natural health foods due to the fact that they are engineered so that the products natural health benefits are enhanced. This is known as biological activity or bioactivity. Bioactivity describes the effect of a compound on the human body either positive or negative, in the case of biological products any negative effects would result in the product being unviable and unsuccessful. The relationship between value and bioactivity can be seen in Figure 1.1. As expected the conventional foods that have no or low bioactivity have a low

market value. As bioactivity increases through fortified foods and health foods their market value increases. This leads to pharmaceuticals, the high level of bioactivity and products with market values into millions of pounds. Although the bioactivity of these sectors increases with increased value, the relative cost of production generally increases with increased amount of bioactivity.

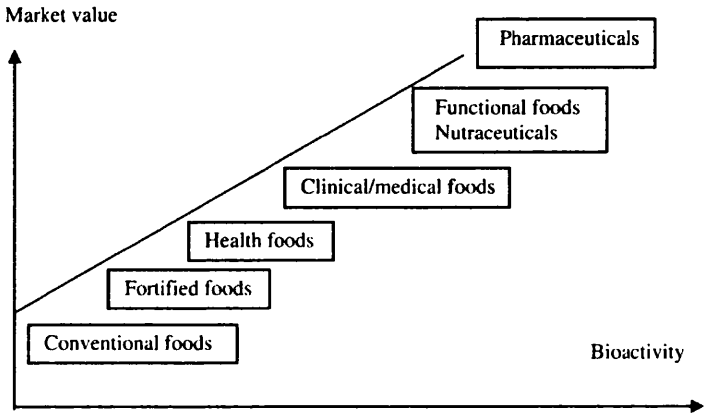

Figure 1.1: The relationship between bioactivity and market value (Korhonen, 2002)

Biological processes have many advantages when compared to solely chemical processes, hence the increase in need for biological product recovery by downstream processing. The driving forces are:

- *Sole route* – Nature is an excellent producer of very complicated molecules, and generally at present the reproduction of such complicated molecules via synthetic processes is difficult, if not impossible. An example is the production of therapeutic proteins or vaccines.
- *Process simplification* – For products that can be produced synthetically, production via a biological route may still be more cost effective and simpler, which potentially could replace several conventional chemical steps, resulting in improved overall process yield. A growing sector is the use of semi-synthetic pathways where a biological product is further processed by conventional chemical processes.
- *Energy saving* – Biological processes often operate at ambient temperatures, pressures and neutral pH.

- *Renewable feedstocks* – Biological processes use carbon derived from glucose as opposed to petrochemicals.
- *Specificity* – Biological processes are often specific for single enantiomers, functional groups or steric positions.

Separation engineers face many similar problems when dealing with biochemical systems, these include:

- *Dilute aqueous solutions* – Water is the solvent for biological processes except in some rare processes. Generally, this makes bioprocessing easier with fewer safety and processing issues to consider, water however tends to have a very high specific heat capacity when compared to other solvents, therefore potential drying costs may be large dependent on bioseparation method selection. The limits of concentration are often dependant on toxicity of the process organism or cell at high concentrations.
- *Complex, multicomponent mixtures* – Fermentations by definition produce a very complex mixture of products. These compounds range from high molecular weight species such as nucleic acids, proteins and lipids, to low molecular weight species such as amino acids, polyphenols, antiobiotics and iminosugars. Furthermore, compounds present may not always be water soluble and may often form colloidal suspensions and particles which further complicates the separation.
- *Poorly defined products* – The number and range of components present in a biological system will be vast, and will have molecular weights ranging from a few hundred Daltons to several hundred thousand. The physical properties (solubilities, isoelectric points, stabilities, pH) of all the compounds present is almost impossible to determine even for a relatively simple process. In addition, the physical properties of the compounds present may be different due to the effect of the other compounds present.
- *Variable composition* – The total number of components present will be very large thus making individual characterisation very difficult. This applies to all biological processes as often the starting feeds differ batch to batch, the ideal scenario however is to maintain close control over any variables.

- *Product stability* - A major factor in the success of a bio product is the stability of the compound of interest. The degradation or loss of bioactivity upon separation or purification would result in an useless product, a phenomena that can affect both low and high molecular weight compounds. The main mechanisms for product loss are chemical, physical or microbial degradation. In the case of chemical degradation, an alteration in pH or temperature may result in the compound becoming denatured. Physical degradation is caused by excessive application of force to the product, for example high pressure being used in membrane filtration, or a high shear rate upon stirring. Microbial degradation, often called spoilage is the degradation through the introduction of enzymes, bacteria etc. that may alter the bioactive compounds through changing the molecular structure or total degradation of compounds into different inactive ones.
- *Multi-technique separations* – A number of steps and differing techniques are often required in order to obtain desirable product purification and quality.

1.2 Bioprocessing unit operations

All biological separations are based on one of the following factors:

Table 1.2: Separation basis and bio-separation techniques of biological separation

Separation basis	Bioprocess separation technique
Size	Filtration, membrane separation, centrifugation
Density	Centrifugation, sedimentation, flotation
Diffusivity	Membrane separation
Shape	Centrifugation, filtration, sedimentation
Polarity	Extraction, chromatography, adsorption
Solubility	Extraction, precipitation, crystallisation
Electrostatic charge	Adsorption, membrane separation, electrophoresis
Volatility	Distillation, membrane distillation, pervaporation

A typical bio-separation process is dependent on whether the bioprocess is reactive or extractive. As stated previously, reactive processing involves biological reactions prior to bio-separation whereas extractive separation almost solely relies on bio-separation. A RIPP (Recovery, Isolation, Purification and Polishing) scheme is commonly used in bio-separations (Mathew and Juang, 2007). A typical bio-separation is shown in Figure 1.2 (Liddell, 1994):

Figure 1.2: General downstream process flow sheet for biological products

Primary separation usually involves a high volume - low resolution separation, with a solid-liquid separation being very common in the case of many extractions and fermentations (biomass). Common primary separation unit operations include centrifugation, filtration (conventional/microfiltration), membrane filtration, flocculation, cell disruption, liquid extraction and adsorption/ion exchange.

The purification stage is very product specific, often with a combination of techniques being used to provide the efficient separation. Typical purification stages include adsorption, solvent extraction, precipitation, distillation and membrane technology.

Product isolation involves the isolation of the purified product, essentially preparing the final product. Techniques that are often used include membrane filtration, evaporation, spray drying, freeze drying, vacuum drying, precipitation, crystallisation, and solid-liquid separation (isolation of precipitates). The boundaries between purification and isolation are quite blurred and often overlap, therefore similar techniques are used at both stages. Depending on the state of the final product an additional drying stage may be required, a stage that may be as important as the previous stages in terms of product stability and quality.

As with all separations the selection of a suitable operation is scale dependent. Primary separation methods are less affected by scale of operation, although

purification processes such as chromatography could be overwhelmed with very large flow rates.

Downstream bio-processing requires the efficient handling of potentially a very large volume and wide range of products due to the diverse nature of potential biotechnological products. The variable nature of biotechnology processes results in variable quantities of products in the feeds combined with varying levels of impurities suggesting that bioseparation processes require thorough investigation in order to select the most efficient separation methods. The diverse nature of potential products means that the scale of separation process is critical in the separation success of any compound. However, bioprocessing often follows a high level pattern of primary separation followed by product purification and isolation to generate the compound of interest. Technological advances in recent years have seen more and more biological products making their way to market, however, the technology utilised in the production processes remains relatively unchanged. An exception to this is membrane technology. Membrane technology, and in particular nanofiltration, has great potential in providing not only a high resolution but, also a high throughput bioseparation. Nanofiltration, as the name suggests, separates compounds in the nano range (~ 1nm / < 1000 MW). Interestingly, 14 of the top 20 best selling drugs in the US market (2007 figures) were small molecules, with the largest selling drug in the world a prime example [Lipitor, Pfizer; a statin for lowering blood cholesterol, MW: 558] (IMS, 2008).

1.3 Membrane Processes

Research into the barrier properties of membranes began at the start of the twentieth century; however the first artificial membrane was not developed for some twenty years (Mulder, 1996). The most significant breakthrough in the industrial application of membrane technology was the development of the first asymmetric membrane in the late 1950s (Loeb and Sourirajan, 1960). Since the late sixties membrane processes have gradually become an integral part of many industrial processes, serving as viable alternatives to more traditional separation methods such as distillation, evaporation and extraction.

Membrane processes offer many distinct advantages over traditional separation processes - highly selective separation; relatively low capital investment and operating costs; low energy consumption; constant temperature operation, with no phase change; continuous and automatic operation; and simple modular construction. These advantages are beneficial to wide range of applications and are especially important for certain types of materials that have been inherently difficult and expensive to separate (Bowen, 1994):

- Finely dispersed solids, especially those which are compressible, have a density close to that of the liquid phase, have high viscosity or are gelatinous.
- Low molecular weight, non-volatile organics or pharmaceuticals and dissolved salts.
- Biological materials which are very sensitive to their physical and chemical environment.

Membranes are able to separate components due to differences in physical and chemical properties between the membrane and the solutes. Transport of both solvent and solute across a membrane is caused by the action of a driving force or driving potential on the feed solution. The possibility exists to classify membrane processes based upon the nature of the driving force or driving potential (gradients in concentration, electrical potential, temperature or pressure) and the physical state of the phase on either side of the membrane.

A classification on this basis of membrane processes is presented in Table 1.3:

Table 1.3: Membrane processes classified by their driving force (Mulder, 1996)

Membrane Process	Feed Phase	Permeate Phase	Driving Force
Microfiltration	Liquid	Liquid	ΔP
Ultrafiltration	Liquid	Liquid	ΔP
Nanofiltration	Liquid	Liquid	ΔP
Reverse Osmosis	Liquid	Liquid	ΔP
Piezodialysis	Liquid	Liquid	ΔP
Gas Separation	Gas	Gas	ΔP
Vapour Permeation	Gas	Gas	ΔP
Pervaporation	Liquid	Gas	ΔP
Electrodialysis	Liquid	Liquid	ΔE
Membrane Electrodialysis	Liquid	Liquid	ΔE
Dialysis	Liquid	Liquid	ΔE
Diffusion Dialysis	Liquid	Liquid	Δc
Membrane Contactors	Liquid	Liquid	Δc
	Gas	Liquid	$\Delta c / \Delta p$
	Liquid	Gas	$\Delta c / \Delta p$
Thermo-osmosis	Liquid	Liquid	$\Delta T / \Delta p$
Membrane Distillation	Liquid	Liquid	$\Delta T / \Delta p$

The majority of the above processes are considered, or are fast becoming considered as proven technologies. The work contained within this thesis will focus on pressure driven membrane processes, therefore no further discussion of the other technologies will be presented. The liquid-liquid pressure driven processes are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). MF membranes typically separate particles in the approximate range of 0.05 - 10 μm such as aggregates, bacteria, biomass, and yeasts at a low operating pressure (ΔP < 2 bar). UF membranes have pore dimensions ranging from 5 – 100 nm and are suitable for the separation of macromolecules (molecular weight ~ 10^4 – 10^6 Da) and colloids such as proteins and enzymes. Initially it was thought that the separation mechanisms involved in UF were predominantly steric but increasingly attention was given to charge effects, which are now considered to play a significant role. The separating layer of UF membranes is denser than that in MF membranes and leads to a larger hydraulic resistance. As a direct result, the operating pressures are greater in UF membranes than MF membranes (1 < ΔP < 5

bar). RO membranes ideally only allow the solvent (in majority of cases water) to permeate the membrane. These membranes are denser still and so the operating pressure must be large ($10 < \Delta P < 100$ bar) to overcome both the hydraulic resistance and the large osmotic pressure gradient (typically the osmotic pressure of sea water is 25 bar). NF membranes have properties that lie between those of UF and RO membranes. Figure 1.3 illustrates the main separation features of the four processes considered.

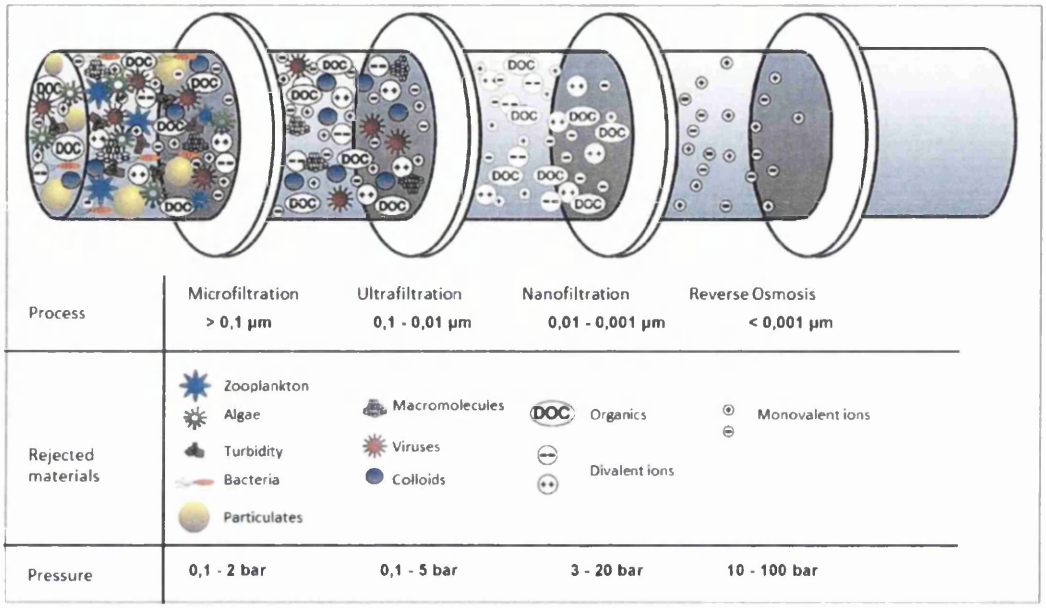

Figure 1.3: The separation features of different liquid-liquid pressure driven membrane processes (figure adapted from original available at http://trinkwasserspezi.de/html/membranverfahren.html).

The membrane construction materials vary dependant on operating size range and pressure. At the higher end of the separation scale (MF and UF) membranes are commonly polymeric, ceramic or even metallic. The materials for the NF and RO membranes are typically polymeric, however, the research and manufacture into other types of membranes such as ceramic has grown in recent years and ceramic NF membranes are commercially available (Inopor membrane range, Veilsdorf, Germany) (Marchetti et al. 2012). Cellulose acetate was originally used throughout the industry; this polymer has now been replaced by a wide variety of polymers which are only limited in diversity by imagination. Common membrane polymers

include polyamides, polysulphone, polyethersulphone, poly carbonate, and polyimide. These synthetic polymers offer improved chemical and mechanical stability and higher resistance to microbial degradation than the original cellulose acetate membranes and are very suitable for use in a wide range of applications. A rapidly growing area is the development of advanced modified polymers for membrane application in organic solvents.

Membrane processes offer a promising alternative for the processing of bioproducts. The development of membrane processes for bioseparations is very similar to the design of non-biological based systems. However, there are notable differences (Zydney, 2000):

- Increased concerns about deactivation or denaturation of biological molecules and cells.
- Very high value (on a per unit mass basis) of most biological products (particularly recombinant therapeutic proteins).
- Tendency of biological macromolecules and cells to cause significant fouling.
- Critical importance of validation and integrity testing in bioprocessing applications.

1.4 Nanofiltration

Nanofiltration (NF) is a pressure driven membrane separation process with characteristics between those of reverse osmosis and ultrafiltration. Nanofiltration was first introduced in the early 1980's and was defined as "a process intermediate between reverse osmosis and ultrafiltration that rejects molecules which have a size in the order of one nanometre" (Eriksson, 1988). Advantages include low operating pressures when compared to reverse osmosis (RO) and higher molecular rejections compared to ultrafiltration (UF) (Stafie et al., 2004). The process has gained popularity due to increased selectivity for mono and multi-valent ions at relatively low capital and operating costs (Cheng et al., 2012; Hilal et al., 2004) particularly as pre-treatment to RO membranes. NF membranes have a nominal molecular weight cut off in the range of 100-1000 Da (Oatley et al., 2012; Cheng et al., 2012; Bessarabov et al. 2002; Baker 2004) and were first developed for use as

an unit operation for water treatment, dairy and chemical industries (Linder & Kedem 2004; White 2006).

The flux characteristics through the membrane are as important as selectivity and therefore most NF membranes are either thin film or composite membranes to minimise hydraulic resistance. The operating pressures used in NF typically ($10 < \Delta P < 40$ bar) are lower than in RO because of the more open pore structure which allows some permeation of solutes, reducing the osmotic potential gradient. UF membranes are often used as a porous support layer to provide mechanical strength. The dense separating or 'active' layer (thickness of separation layers are typically 100 nm but can be as low as only 1 – 2 nm in some cases), which is assumed to control all separation characteristics, is normally deposited on the UF support using either dip coating or interfacial polymerisation.

Laboratory based studies with NF membranes almost always use flat sheet membranes either in dead end or cross flow mode, this is mainly due to the feed volumes used at a research stage. Although pilot scale studies often use industrial scale membranes one module would be used as opposed to banks of modules which would be utilised on an industrial scale. Industrial NF modules can be configured in tubular, hollow-fibre or spiral-wound geometries. The last of these configurations is often used because the high packing density (300 – 1000 $m^2\ m^{-3}$) allows for greater filtration areas than tubular membranes and higher fluxes than hollow-fibre membranes. The development of new membrane materials is leading to the development of more efficient hollow fibre and tubular membrane particularly for high fouling materials. The spiral-wound membrane configuration is prone to fouling and requires careful pre-treatment for feed streams that contain potential fouling materials. Flat sheet membranes are arranged around a central permeation collection tube in a Swiss roll arrangement (Figure 1.4) with the membranes being separated by spacers and turbulence promoters, the properties of which are vital when considering potentially fouling or heavy solid loading materials.

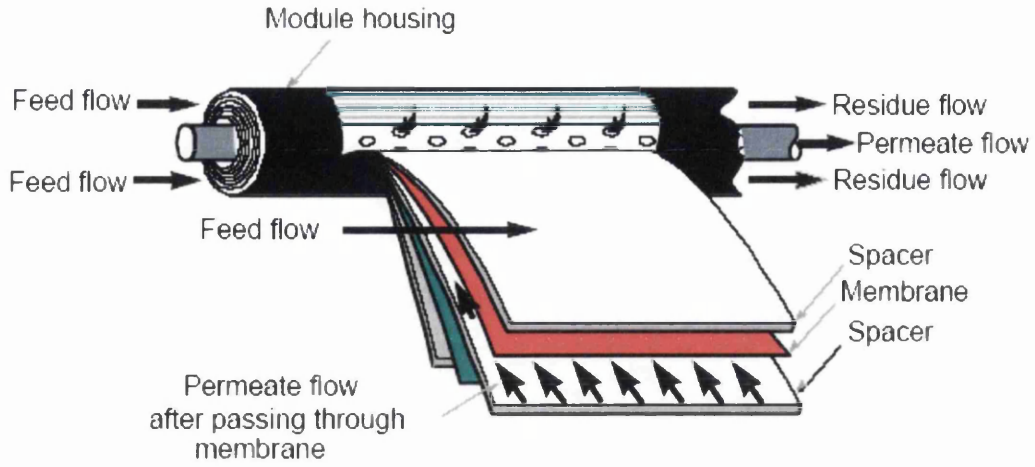

Figure 1.4: A spiral wound membrane module (Oatley, 2004).

The selection of membrane material is highly important in NF and can influence the separation characteristics of the membrane. The presence of ionisable groups in the active layer provides the membrane with an ionic charge. These charges can either be positive (formed from cationic groups such as NH_4^+) or negative (formed from anionic groups such as $COOH^-$); most NF membranes tend to be negatively charged.

1.5 Separation mechanism

Traditionally, membrane processes, and in particular NF have been designed and developed using a series of membrane trials in order to achieve the desired separation. Although membrane trials result in an acceptable process, the practice is often expensive and time consuming. The development of good predictive models is therefore essential in both process design and to understand the intricate details of the complex NF process. Understanding the factors that affect the separation properties of NF membranes is very important for both laboratory and industrial engineering applications. A well developed predictive model must account for the following factors:

- The NF membrane itself – structural parameters such as pore radius and membrane thickness, electrical parameters such as charge density and other factors such as degree of fouling and polymer swelling.

- The feed solution – characteristics of ions or solutes, concentration, pH and fouling potential.
- The operating unit – capacity, dimensions, flow rate, mass and heat transfer parameters.
- The process environment – temperature and pressure.

The last two factors involve fundamental principles of chemical engineering and are elaborated in great detail in various chemical engineering text books (Sinnott, 2005; Green and Perry, 2007). The first two factors, which are specific to the NF membrane system are interrelated and are very important in understanding the separation behaviour in NF systems.

The development of NF membranes was driven by the requirement to process the range of materials that pass freely through UF membranes while being fully retained by RO membranes. Conventional RO membranes reject almost all solutes while UF membranes are used for the concentration and separation of colloids, proteins and other relatively large macromolecules. As a result, the pore sizes in NF membranes were designed to make them very effective in the separation of uncharged and polar organic solutes with molecular weights in the range 100 – 1000 Da.

NF differs from other pressure-driven liquid phase membrane processes by potentially being able to separate molecules of similar size. The combination of small pore size and 'fixed' membrane charge allows the separation of nano-range organic molecules and ions based of differing valances (Hilal et al., 2005). Rejection for monovalent salts and uncharged solutes with molecular weight <150 Da is typically low, while divalent and multivalent ions and organic with molecular weight > 200 Da is typically high (Kelewou et al. 2011). Separation in NF is governed by both size exclusion and electrostatic interactions, although typically uncharged solutes are separated purely by steric mechanisms. Therefore, the separation and rejection of ions in the NF range must be attributed to both steric and non-steric effects (Yaroshchuk, 2001; Szymczyk & Fievet, 2005).

Membrane nanofiltration is extremely complex and is dependent on the micro-hydrodynamic and interfacial events occurring at the membrane surface and within the membrane nanopores. The rejection mechanism takes place in pore dimensions of approximately 1 nm (Oatley et al. 2012), a scale not much greater that atomic dimensions, leading to separation mechanisms not previously encountered with UF.

Higher rejection of higher valance ions was originally attributed to equilibrium partitioning at the entrance and exit of the NF pores. Initial work was based solely on the Donnan exclusion principle, where the efficiency of ion exclusion is dependent on the valance of the counter ion increasing or decreasing (Donnan 1911). As an example, for a negatively charged membrane, rejection of salts with divalent anions such as Na_2SO_4 is always high compared to monovalent salt such as NaCl.

In addition to the charge effect, Tsuru et al. (1994) discussed the importance of the steric mechanism in the partitioning of ions. Bowen and Mukhtar (1996) included steric effects in their analysis of salt rejection. Further work by Bowen et al. (1997) and Bowen and Mohammad (1998) investigated the effect of ion size for the nanofiltration of LiCl, NaCl, and KCl, reporting the order of rejection follows KCl>NaCl>LiCl and consequently the same for the counter-ions, which is explained by the decreasing diffusivities. Interestingly, later research undertaken into salt-sugar solutions reported a reduction of NaCl rejection when the sugar-salt concentration increases (Vellenga and Tragardh, 1998). However, when the topic was revisited by Wang et al. (2002) the conclusion was that NaCl concentration affects the rejection of glucose while the rejection of NaCl in independent of glucose concentration, a phenomena explained by Bargeman et al. (2005). The effect observed was attributed to an increased concentration of charged solutes resulting in greater membrane surface charge within the pores and hence, a pore swelling effect, an argument supported by Mohammad et al. (2010). In contrast, Bouchoux et al. (2005) hypothesised that an increased salt concentration leads to less hydration of the glucose molecule and therefore results in a smaller molecule and hence lower rejection.

The application of steric and Donnan theories alone cannot predict the high rejection observed for binary electrolytes with divalent counter-ions. The Donnan Steric Partitioning Model (DSPM) model could account for the behaviour observed, however, the magnitude of membrane charge required was unrealistic (Bowen and Welfoot, 2002). Therefore an additional phenomena was required in order to account for the observed transport. This lead to the application of dielectric exclusion in order to explain the interactions between ions with polarisation charges that are induced at the solvent-membrane interface (Yaroshchuk, 2000), descriptions covering dielectric exclusion include those considering the phenomenon of image forces. This effect is dependent on the square of the ion charge, and irrespective of the sign of the charge, the rejection of divalent ions was greater than that of monovalent ions.

Bowen and Welfoot (2002) proposed that the dielectric exclusion could be attributed to the solvation energy barrier formed when an ion passes from a solvent of one dielectric constant to a solvent of a different dielectric constant. The unique spatial confinement of a NF pore results in a spatial reorientation of the solvent molecules compared to that of the bulk solution. The spatial rearrangement of the solvent into discreet layers directly affects the physical and electrical properties of the solvent, thus leading to a change in the dielectric constant. This behaviour was originally described by the Born model (Born, 1920) and was again dependent on the square of the ion valence.

At present, the separation characteristics of NF are attributed to the following three mechanisms (Deon et al. 2011; Montalvillo et al. 2014):

- Steric effects - related to the relative size of the solute and pore.
- Electrostatic (Donnan) effects - can be either attractive or repulsive dependent on the valance of the ion and sign and magnitude of the fixed membrane charge (usually negative for NF membrane (Cheng et al. 2012)).
- Dielectric interactions - where multivalent ions are rejected to a higher degree than monovalent ions due to interactions between the ions, membrane and solvent at the surface and inside the NF pores.

1.6 Industrial applications of nanofiltration

NF membranes have found an ever increasing number of applications in an ever widening range of industries over the past 25 years. Unlike RO, the scale of application of nanofiltration is difficult to estimate due to the diversity of applications. The use of RO for the desalination of sea and brackish water outweighs all other applications of RO making for easy estimation of worldwide installed RO capacity. Although NF often finds application as a pre-treatment for RO systems the overall diversity of the application and varying installed capacity makes an accurate estimate of worldwide installed capacity almost impossible, however the years 2007 - 2012 saw over 1000 academic articles being published covering NF research (Mohammad, 2013).

The work contained within this thesis predominantly concentrates on biological applications of nanofiltration which will be discussed in greater detail in Chapter 2; Table 1.4 lists some of the most recently reported applications of NF membranes:

Table 1.4: Recently reported applications of nanofiltration

Application	Reference
Water treatment	
Rubber industry effluent	Xin et al. (2013)
Textile industry effluent	Liu et al. (2011)
Drinking water production	Kim et al. (2007)
Removal of pesticides	Musbah et al. (2013)
Removal of pharmaceuticals from drinking water	Vergili (2013)
RO pre-treatment	Song et al. (2013)
Colour removal	Ong et al. (2014)
Car wash water recovery	Boussu et al. (2007)
Radioactive material removal	Zakrzewaska-Trznadel (2013)
Food and Biotechnology	
Fermentation wastewater	Liu et al. (2013)
Endocrine disruptor removal	Yuksel et al. (2013)

Edible oil	Firman et al. (2013)
Pharmaceutical removal	Miralles-Cuevas et al. (2014)
Desalination of soy sauce	Luo et al. (2009)
Dairy whey demineralization	Pan et al. (2011)
Clean-in-Place (CiP) water recovery	Kaya et al. (2009)
Fruit juice concentration	Warczok et al. (2004)
Beverage industry	Pan et al. (2013)
Pharmaceutical Separation	Homayoonfal and Mehrnia (2014)
Fish amino acid separation	Saidi et al. (2014)
Bone collagen dating	Boudin et al. (2013)

The applications listed cover only a small sample of the nanofiltration market, which is very diverse. The applications described in Table 1.4 are predominately water based; recent developments in the materials used to produce NF membranes has opened up a whole new sector, organic solvents. This emerging technology, predominantly called organic solvent nanofiltration (OSN) has received a great deal of attention in recent years. Literature suggests that the application of NF in non-aqueous sectors will experience the greatest growth in the coming years, OSN therefore will be discussed further in Chapter 2.

1.7 Objectives of the present work

This thesis attempts to apply membrane technology, particularly nanofiltration, for the separation, recovery and purification of small molecule bioactive compounds.

Traditional bio-separations are heavily solvent dependant. Membrane technology and in particular nanofiltration, potentially offers a viable alternative for the separation and purification of bioactive compounds. The primary purpose of this research is to attempt to apply nanofiltration as an alternative to traditional bio-separations.

The present work is also intended to contribute towards the understanding of NF membranes and processes through the application of existing NF theory to experimental modelling work. The experimental modelling work attempts to

confirm assumptions and theories proposed for NF separations, confirming work undertaken previously and attempting to close the void between actual industrial NF bio-separations and theoretical understanding.

Furthermore, a collaboration was initiated with the Biocontrol and Natural Products (BaNP) group of Swansea University investigating the application of membrane technology for the separation and purification of fungal bioactives for use in pest control.

Finally, a collaborative research project, with PhytoQuest Ltd. (Aberystwyth, UK) has provided an opportunity to evaluate the scientific and engineering challenges of applying membrane technology to a novel separation. Both laboratory scale feasibility experiments and pilot scale production experiments were investigated.

These overall objectives will be achieved by:

a) Investigation of the mass transfer characteristics of three frontal filtration membrane cells and subsequent comparison of experimentally derived data to theoretical correlations.
b) Experimental determination of nanofiltration hindrance factors through the physical measurement of membrane pore size and solute size followed by a series of rejection experiments. Comparison of hindrance factors obtained to theoretical descriptions in order to ascertain validity of models for the description of a nanofiltration process.
c) The investigation of the current best practice in experimental nanofiltration modelling by applying the extended Nernst-Planck equation to a concentrated salt solution at the membrane's iso-electric point. Specifically investigate the role of dielectric exclusion in nanofiltration separations, and the separation of a concentrated salt solution.
d) Investigate the use of nanofiltration for the isolation of bioactive compounds from a fungal source with a comparison to the traditional solvent extract process, paying particular attention to membrane separation performance and subsequent bioactive properties of the fraction obtained.

e) Finally, a feasibility study for the use of membrane technology for the separation and purification of calystegines from waste potato peel. The initial work will aim to study the compositional changes in potato peel with storage time in order to produce the optimum feed. A laboratory based membrane study will be undertaken in order to optimise the process prior to performing a full scale pilot trial in order to assess the feasibility of using membrane technology for the industrial recovery of calystegines.

2.0 General Nanofiltration Literature Review

This chapter provides a general review of the recent developments of nanofiltration. The chapter covers the applications of nanofiltration in food processing, pharmaceutical separations and biotechnology applications. A brief overview of the relevant membrane theory is also provided.

2.1 Overview of membrane technology

The importance of separation technology is often underestimated; efficient separations are crucial in the field of engineering. A typical chemical/bio-chemical process often consists of a reaction/fermentation followed by a variety of separation methods for purification, extraction or concentration prior to sale of the product.

Obtaining high quality, pure products is essential to all industries, ranging from oil and heavy chemicals to food, water and pharmaceuticals. With greater emphasis placed on process cost reduction, due to increasing economic pressures, and with an increasing requirement to develop sustainable processes, to meet government environmental targets, the need for more efficient and effective separation techniques has increased in recent years. Historically a variety of processes have been developed and used including distillation, precipitation, crystallisation, extraction, adsorption, ion-exchange and evaporation. These methods have been widely used and have provided efficient and effective, although energy intensive, separations.

Bioactive natural products are the main source of new drugs, functional foods and food additives. More often than not they are usually secondary metabolites of plants and animals often generated by a wide variety of biological pathways. Bioactive products provide many beneficial features not always found in solely chemical compounds; these include (Ren et al. 2013):

1. Complex, diverse structures – often unfeasible or impossible to replicate synthetically, examples include flavonoids, alkaloids, sterols, terpenes, and iminosugars.

2. Molecular mass between 200 and 1000, usually containing a variety of complex aromatic rings – Consequently, potentially making NF membrane separations an ideal processing method.
3. Wide range of physiological activities.

"Medical interest in drugs obtained from plants has led to an increased need for an ideal extraction method, which could obtain the maximum of the bioactive constituent in a shortest processing time with a low cost" (Conidi et al. 2011).

The potential of membrane technology in the separation and purification of biological compounds is unquestionable, with the potential to facilitate in the cost-effective separation of a wide range of biological compounds (Zydney, 2000). A review of membrane separations in biotechnology was undertaken by van Reis and Zydney (2001; 2007) as well as by Charcosset (2006). However, each article only superficially discusses the application of nanofiltration as a separation technique indicating the relative youth of the technology in biological separation (Reis and Zydney, 2001). Nanofiltration has been widely used for drinking water production (Hilal et al. 2004) mainly concerning desalination, however recent years has seen the growth of nanofiltration for the specific removal of a variety of synthetic organic micropollutants (pharmaceuticals, detergents, disinfection by-products, steroids, plasticizers, endocrine disruptors) (Plakas and Karabelas, 2012)

The largest issue facing the widespread application of the technology is the flux decline with process operating time. This flux decay, mainly caused by fouling and concentration polarisation, leads to shortened membrane life, variable separation efficiency and increased processing costs (Ren et al. 2013). Fouling, or in this case bio fouling, is the build up of natural organic matter (NOM) on the membrane active surface, and is one of the most commonly reported membrane problems (Fonseca et al. 2007; Vrouwenvelder et al. 2009; Nghiem et al. 2010; Botton et al. 2012).

The global market for nanofiltration increased from $89.1 million in 2006 to an estimated $310.5 million in 2012, with water treatment accounting for approximately 72% of the total revenues in 2007 (BCC Research LLC. 2007). A

market report by Frost and Sullivan (2008) expects the nanofiltration market to be worth $2.2 billion by 2020. This suggests significant growth; however, the greatest issue will inevitably be utilising research data and implementing nanofiltration on an industrial scale. Application of membrane technology for water treatment is successful; however, the potential for biological separations is significant. Developments in the application of nanofiltration for biological and food separations will be presented in this chapter.

2.2 Food Separations

Chemical Engineering has had a profound effect on food production, including advances in fertilisers, improved packaging and development of sophisticated separation techniques such as membrane technology (Rodowicz, 2009). The use of membranes in the food industry has been widespread for many years, driven by the ever-changing needs of the consumer. Sharpe and co workers studied the factors affecting membrane filtration of food suspensions as far back as 1979 (Sharpe et al. 1979). Safety and quality improvement, high nutritional value, consumer convenience and acceptance, product and processing costs always have and continue to be fundamental aspects of process design particularly in the food sector (Lazaridesa, 2011). The use of nanotechnology in the food industry has become established in the last decade with the United States Department of Agriculture (USDA) officially addressing the issue in a 2003 roadmap (Joseph and Morrison, 2006; Cushen et al. 2012). Nano-techniques and nano-foods are being developed in order to assert greater control over food characteristics such as flavour, texture, speed and efficiency of processing, heat tolerance, shelf life, traceability, safety, bioavailability of nutrients and cost efficiency. The introduction of nanosciences and nanotechnologies to food applications offers a wide and varied range of benefits to the consumer (Chaudhry and Castle, 2011). The development of nanotechnology for food is of particular interest in the case of functional foods, as they allow the manipulation of properties close to a molecular level (Momin et al., 2013). The term 'nanofood' covers any food product that has been cultivated, produced, processed or packaged using nanotechnology techniques, or to which any nanomaterials have been added (Sozer and Kokini, 2009; Momin et al. 2013). Any use of nanofiltration

in food production processes would result in a 'nanofood'. Nanofiltration in the food industry may serve simply as a cost effective alternative to RO in food production, however, NF technology has already found uses in desalination, demineralisation, whey and juice filtration, colour removal, and water purification (Warczok et al. 2004; Butylina et al. 2006; Galaverna et al. 2008; Walha et al. 2008; Luo et al. 2009; Suarez et al. 2009; Pan et al. 2011; Walha et al. 2011; Prudencio et al. 2014; Andrade et al. 2014).

With the countries within the European Union producing 89 million tonnes of food waste per annum food wastes present not only a resource challenge but is also an environmental and economic issue (Mirabella et al. 2013). New governmental initiatives are aiming towards a 'zero waste' society where all wastes are used as raw materials for the production of new products and applications. The discarding of food waste is a significant loss of valuable materials, materials which often have the potential for application in other areas e.g. bio-refineries. Traditionally the processing of food wastes was considered a necessary step before discarding, often to meet water discharge specifications. However, in recent years the true potential value of food wastes has been appreciated (Aruoma, 2010).

The first step for valorisation of the food processing sector is to identify, quantify and characterise food wastes and by-products (Rosentrater, 2005). Following identification of potential products and added value components, the exploration of recovery and purification stages can then be undertaken. The recoveries of such compounds require the application of both conventional and novel technologies (Galanakis, 2012). Mirabella et al., (2014) have thoroughly reviewed the options available for the reuse of food manufacturing waste. A review of nanofiltration applications in the food industry was undertaken by Salehi (2014), where the application of the technology to water softening, wastewater treatment, beverage, dairy and sugar processing industries were covered.

2.2.1 Dairy Industry

The dairy industry has been using membrane technology since the late 1960's (Jiao et al., 2004) making the dairy sector, along with the water industry, the first to

utilise membranes on a large industrial scale. Traditionally membranes were applied to the treatment of whey although technological developments have seen the introduction of the technology to many other aspects of the dairy industry (Pouliot, 2008; Salehi, 2014).

The production of cheese results in large volumes of waste water that often has a high Chemical Oxygen Demand (COD), resulting in necessary treatment before disposal. The whey waste product contains significant quantities of valuable components such as lactose and protein, from which valuable by-products can be produced through successful recovery. NF can effectively recover these components while simultaneously reducing the COD of the waste water. Studies into using NF as a treatment for whey effluents in the dairy industry have been carried out since the mid 1990s (van der Horst et al. 1995; Alkhatim et al. 1998) when NF technology was in its infancy. Research into NF as a treatment method continues. Pan et al. (2011) investigated the effect of acidification of the waste whey on the desalination using a pilot scale TFC 2540 SR2 NF membrane module. The work undertaken showed optimum rejection of protein at the whey isoelectric point (pH 4.6), at which point the solubility of the protein is lowest (Pan et al. 2011) and essentially rejecting a 'neutral species'. The demineralization rate was reported as 72%. The work by Pan et al. (2011) furthered work undertaken by Suarez et al. (2009) where an automated NF pilot plant containing a polyamide DK2540C membrane (MWCO: 200) was used. The work used NF to partially demineralise ultrafiltration permeate and whey, with results reporting that when whey is directly nanofiltered a flux reduction is observed owing to a gel layer build up. Higher mineral salt removal is observed in the ultrafiltered feed, with 36% removal observed in the ultrafiltered feed when compared with 27% salt reduction in the whey feed. Further work on the nanofiltration of whey production permeates was undertaken by Butylina et al. (2006), with the work focussing on the recovery of valuable peptides present in the permeate after the production of sweet whey using a 10kDa ultrafiltration membrane. The work used a 2.5 kDa membrane for filtration followed by a NF membrane (NTR-7450 MWCO:1000) for purification. The membrane was selected as the MWCO allowed for the separation of the lactose

from the polypeptides present. The fouling of the membrane altered the purification results, with a higher fouling reported at both high and low pHs. A selectivity of 0.82 was observed at high pH compared to 6.5 at low pH suggesting the purification was optimum in acidic conditions despite the generation of a fouling layer. Butylina et al. (2006) summarised that a polypeptide separation can be undertaken using a hybrid membrane process although the process would require optimisation dependent on the peptides of interest, and furthermore would require optimal selection of membrane and operating conditions. The rejection of lactose by nanofiltration from a sweet whey feed was modelled by Cuartas-Uribe et al. (2007) who applied two predictive nanofiltration models, namely the Kedem-Spiegler (KSM) and Donna Steric Partitioning model (DSPM), to the separation. The KSM model produced better predictions for the separation of lactose, however the KSM model only considers solute permeabilities and reflection coefficient compared to the DSPM which considers convective, electric and diffusive transport. The separation of the uncharged solute lactose in this case suggested that the simplest KSM successfully predicted the separation in both the presence and absence of ions. As previously mentioned (Wang et al. 2002), the presence of ions can reduce the observed rejection of the uncharged solute, a phenomena supported by Cuartas-Uribe et al., (2007).

Prudencio et al., (2014) investigated the use of NF (HL2521TF membrane, MWCO: 150 - 200) and NF-diafiltration on the treatment of whey waste for the production of ricotta cheese. The pilot scale study reported no noticeable differences in the composition of the retentates of the two processes, however the study did note that the membrane processes resulted in more elastic, firmer and more compact ricotta cheese. A similar study for the production of cottage cheese was reported by Nguyen et al., (2003).

2.2.2 Food Ingredients

Luo et al. (2009) used four commercial NF membranes for the desalination of raw soy sauce due to increasing consumer demand for lower sodium levels in foods. Traditionally a number of technologies have been used for the desalination of soy

such as ion exchange, reverse osmosis, electro dialysis, freeze concentration and dialysis. However, these technologies have had an adverse effect of the soy sauce, with disadvantages such as poor flavour and high capital and operational costs being reported. Conversely, the removal of 50 % of the salt content by NF has been reported as having had no detrimental effect on the taste and flavour of the soy sauce leading to the belief that NF is a feasible technology for soy sauce desalination (Luo et al. 2009). The work undertaken found the NF 270 membrane optimum for the desalination process, with advantages such as high flux and relatively low loss of nutritional components. To preserve product quality, the authors reported a solution-concentration-diafiltration as the optimum processing method, offering high salt removal, high nutrient retention, low water consumption and short processing time.

Food production generates a large volume of waste water, which often contains agricultural solid wastes and high Biological Oxygen Demand (BOD), both of which present a disposal problem. Recent years has seen interest in valorising these waste streams grow. Compounds now found to be of bioactive interest have often been discarded. Artichokes have displayed a number of interesting pharmacological properties for a variety of ailments and their production on an industrial scale produces large quantities of waste water. The production of a saleable product results in the partial transfer of the bioactives from the artichoke to the wash and processing water and therefore results in a potentially valuable stream. Conidi et al. (2014) investigated the application of a combined UF/NF system for the removal of solid followed by the concentration of the artichoke phenolics. The experimental work concluded that the appropriate selection of NF membrane is vital for process success. The two NF membranes (NP030, Desal DL) tested both displayed high rejection of phenolics although the sugar retention with the NP030 membrane was low (4%) when compared to the Desal DL membrane (100%). The authors concluded that the retentate fraction form the NP030 membrane had potential in the cosmetics, nutraceutical and food industries while the sugar enriched Desal DL fraction had potential in the food industry as an additive. Furthermore, the purity of

the Desal DL permeates (containing water and salts only) allowed for recycling either as process water or for cleaning purposes.

The aroma of food and beverages is often a key selling point and a sign of product quality, therefore, recovery of aroma compounds during processing is vital. The term aroma covers a large range of substances; according to Pereira et al. (2005) the number of aroma compounds detected in fruit alone exceeds 6000. The recovery of aroma compounds from tuna cooking brine by NF was attempted by Walha et al. (2009) and again by Walha et al. (2011) using a one and a two step process. The work undertaken found that the implementation of a MF prefiltration stage resulted in the enhanced performance at the NF stage. The process developed successfully concentrated the marine aroma contained in the highly salted cooking juices.

Further examples of applying NF to the beverage industry can be seen by Vincze and Vatai (2004) and Pan et al. (2013), both of which applied NF as a method for the concentration of coffee extract in the production of instant coffee. The original work by Vincze and Vatai (2004) increased the concentration of the coffee extract from 14 to 45 g/l. The work was furthered by Pan et al. (2013) who suggested that a greater concentration could potentially be obtained and that the original results obtained by Vincze and Vatai (2004) did not cover membrane performance at high coffee extract concentration. Pan et al. (2013) furthered the research by applying six commercial NF membranes for the concentration process and concluded that the NF-2 membrane produced by SEPRO was the most efficient. The work by Pan et al. (2013) theoretically predicted a maximum concentration of 39% at 40 bar compared to 4.5% in the previous work, stating that the 39% value was too low to directly enter spray/freeze drying process and ultimately would require further concentration, possibly by conventional evaporation. The work undertaken thus far has utilised NF as a processing method for the partial concentration of coffee extract in the production of instant coffee, however coffee contains a vast range of bioactives such as phenolic compounds (Murthy and Naidu, 2010), and therefore in future NF may be applied for this purpose.

2.2.3 Juice Processing

The application of membrane technology for the production of concentrated juices has been prevalent for over a decade (Jiao et al. 2004). The task of concentrating juices has often been designated to RO systems, due to the high rejection of almost all solutes except water, making an ideal concentration technology when compared with harsher techniques such as evaporation (Madaeni and Zereshki, 2010). As with the majority of biological sources recent years has seen an increase in fruit, and specifically tropical fruit, consumption due to the increasing recognition of the high nutritional and therapeutic value (Rufino et al., 2010). A combination of membrane technologies is often employed for the concentration of juices in order to minimize losses of flavour and nutrients during processing. The concentration of fruit juices reduces the volume which consequently reduces the costs of transportation, storage and packaging (Sotoft et al., 2012). Concentrated juices offer increased shelf life and a higher resistance to microbial and chemical spoilage due to the reduced water quantity (water activity). A combinatory method for the concentration of blackcurrant juice was designed by Sotoft et al. (2012) where a combination of RO, NF and membrane distillation was used, resulting in a process 43% cheaper than a traditional concentration process. Nanofiltration has also been used as a method for juice concentration by other workers (Versari et al. 2003; Warczok et al. 2004; Banvolgyi et al. 2006; Vincze et al. 2007).

The application of membrane technology for the processing of fermented drinks is a controversial topic especially when applied to wine processing due to the effect filtration has on colour, aromatic profile and polyphenol profile (Arriagada-Carrazana et al. 2005). Despite this, the application of nanofiltration for the concentration of wine components is increasing, with membrane filtration becoming established for the clarification of wine and beer (Salehi, 2014). An overview of the application of nanofiltration and reverse osmosis in winemaking was presented by Massot et al. (2008). The article discussed the use of NF to control alcohol content in wines, control of acidity and removal of 'bad taste' compounds. Ferrarini et al. (2001) suggested the use of NF and RO as a realistic alternative to pervaporation and cryoconcentration for the concentration of grape

juice. The production of wine requires a high level of control over the grape must, as grape must composition is essential to alcohol content and quality. The addition of additives (ethanol, sugar etc.) is one potential method, however many consumers see such a process as artificial, therefore other methods for concentration of existing grape musts have been investigated with nanofiltration one of them. Versari et al. (2003) used nanofiltration in order to concentrate the sugar content of grape must, finding that NF provided a suitable concentration achieving a product of similar composition to the initial grape must. Conversely the work undertaken by Garcia-Martin et al. (2010) attempted to reduce the sugar content in grape must for the production of low-alcoholic wines. The process designed used a two stage NF process for the sugar reduction reportedly producing a lower-sugar must with satisfactory sensory qualities, a quality often lost when processing. Catarino and Mendes (2011) studied the application of reverse osmosis and nanofiltration for the dealcoholisation of 12% red wine. The research studied four NF membranes with similar properties, all operated in diafiltration mode, and followed by a pervaporation step for the recovery of aroma compounds prior to reconstitution to produce the final product. Three NF membranes (YMHLSP1905, NF99, NF99 HF) performed well due to their high permeability to ethanol combined with high rejection of aroma compounds. The authors noted that the results obtained showed promise in the use of a combined NF-pervaporation process for the dealcoholisation of wine. The dealcoholisation of a model white wine solution was studied by Labanda et al. (2009) using RO and NF membranes reporting efficient removal of ethanol and less than 15% removal of each of the flavour compounds.

2.3 Membrane processes in biotechnology

Membrane processes are increasingly being used for unit operations such as separation, clarification, purification and concentration of molecules, emulsions and particles, particularly in the biotechnology sectors. Generally speaking membrane technology is well suited for the processing of biological systems since they operate at relatively low temperatures and pressures (membrane process dependant) and involve no phase change or chemical additives, therefore

minimising the extent of denaturation, deactivation and/or degradation of the biological products (Zeman and Zydney, 1996; Ren et al. 2013). A review of the application of membrane technology was carried out by Saxena et al. (2009) for the separation and purification of protein; the size range of proteins however often negates the use of NF.

2.3.1 Biological Applications

Sjoman et al. (2007) applied nanofiltration for the separation and purification of a monosaccharide mixture, in this case xylose and glucose. Monosaccharides, usually purified by chromatographic methods, are widely used in the food and pharmaceutical industry, therefore the production of a pure fraction is essential. The separation of sugars is more difficult as the solutes are uncharged and therefore the separation is based upon differences in molecular weight and diffusivities alone. The work undertaken by Sjoman et al. (2007) used three commercial NF membranes (Desal DK, Desal DL, and NF 270, All MWCO: 150 – 300 Da) for the separation of the solutes. The separation was performed with limited success, with the three membranes studied giving comparable fractions indicative of a size based separation. The results obtained strongly suggested that separation was dependent on permeate flux and hence feed pressure, with feed concentration having no effect of separation performance. Additional research into xylose separation was undertaken again by Sjoman et al. (2008), where xylose purification from hemicellulose hydrolyzate feeds for the production of xylitol, an artificial sweetener. A similar example was the extraction and concentration of monosaccharides and non-saccharide compounds (glucose, arabinose, xylose, and acetic acid) from rice husks, highlighting the dilemma that a membrane process is often a trade-off between recovery and purity. However, Vegas et al. (2008) noted that in order to increase purity further processes may be combined with membrane technology such as ion exchange, similar to the argument put forward by Tsibranska and Saykova (2013).

Weng et al. (2009) utilised a model solution for studying the removal of acetic acid from xylose, for the purpose of improving bio-ethanol production, reporting a

separation heavily influenced by pH achieving a maximum separation factor of 5.4 at a pH of 2.9. Weng et al. (2010) furthered the research into bio-ethanol production by using NF for the separation of furans and carboxylic acids from sugars in dilute rice straw hydrolyzates using a Desal DK membrane at the conditions optimised in the their previous work; this time a separation factor of 49 was achieved for the separation of acetic acid over xylose. The separation of acetic acid using NF technology was also studied by Teella et al. (2011), although in this case the separation was from a bio-oil produced by fast pyrolysis (rapid heating of biomass in the absence of oxygen). Teella et al. (2011) highlighted that much of the previous work undertaken into the NF separation of carboxylic acids from biological sources have used fairly low concentrations or fairly simple mixtures of organic compounds (Sjoman et al. 2007 ; Weng et al. 2009; Weng et al. 2010). The work using a model Bio-oil solution resulted in irreversible damage to the membrane polymer due to the presence of guaiacol; however when this compound was removed glucose rejections of up to 80% were observed in the model solution. Teella et al. (2011) concluded that a membrane process for the separation of low molecular weight organics from sugars was possible although the process would require a resistant polymeric membrane and relatively high trans-membrane pressures in order to achieve sufficient flux and separations.

As stated previously, nature provides an excellent source of complex bioactive compounds, compounds which would not be studied if they could only be produced synthetically. An example of which are the class of chemicals know as phenols, which have displayed a variety of interesting bioactive properties. In 2010 both Tylkowski et al. (2010) and Mello et al. (2010) both produced research into the extraction and concentration of flavonoids and phenolics from propolis, which is a resinous natural product produced by bees. Tsibranska et al. (2011) and Tylkowski et al. (2011) investigated the extraction of polyphenols and flavonoids from *Sideritis* ssp. L. plant (flowering plant found in the Mediterranean) using solid-liquid extraction and membrane technology respectively. The paper by Tsibranska et al. (2011) optimised the extraction process, extracting 90% of the phenolics present in the first 2.5 hours. The work was then furthered by Tylkowski et al. (2011) where

the extract was concentrated through the use of organic solvent nanofiltration. The authors reported total rejection when a 300 Da membrane was used, obtaining a 3-4 times concentration and subsequently reporting a increase in biological activity (antioxidant activity).

The increasing interest in replacing synthetic antioxidants has encouraged much research in biological sources, which are seen as a safe and natural source. The concentration of antioxidants was studied by Machado et al. (2013) who applied NF technology to the Brazilian fruit pequi. The results published state that ethanol extracts more carotenoids whereas water extracts more polyphenols, with both extracts displaying high antioxidant activity. However, the concentration of the ethanolic solution by NF resulted in poor retention of the bioactive compounds, suggesting further work is required for the concentration of pequi extracts by nanofiltration.

Similar research was carried out by Murakami et al. (2011) and Prudencio et al. (2012) who both investigated the recovery and concentration of phenolics from mate (commercially important plant in South America). The native Chinese tree Ginkgo Biloba is known to contain many different glycosides and terpenoides which exhibit a wide variety of biological activity, which includes use in the treatment of Alzheimer's disease. The membrane process applied for the concentration of Ginkgo Biloba extract rejected 99% of the bioactive compounds therefore providing a suitable alternative to the traditional low-temperature and three-effect evaporation process (Xu and Wang, 2005).

The extraction of biological compounds from plant materials is ever growing and diverse, many of the attempts to recover and purify such compounds often use similar technologies. Table 2.1 highlights the use of nanofiltration for the extraction, purification and concentration of bioactive compounds from biological waste sources:

Table 2.1: Biological uses of nanofiltration reported in the literature

Application	Source	Extraction Medium	Membrane	Reference
Isoflavones	Soybean	Water	PVDF	Benedetti et al. (2013)
Antioxidants	Grape pomace	Press liquors	DL2540, Nanomax 50, Nanomax 95	Diaz-Reinoso et al. (2009); Diaz-Reinoso et al. (2010)
Anthocyanins	Roselle extract (*Hibiscus sabdariffa* L.)	Water	NF90, NF200, NF270, UTC60, MPF36, MPF34, Desal DL, Desal DK, NP010, NP030	Cisse et al. (2011)
Anthocyanins	Orange press liquor (Blood Orange)	Press liquors	N30F, NFPES10, NF70, NF200	Conidi et al. (2012)
Peptides	Marine processing waste	-	-	Harnedy and FitzGerald (2012) Review Paper
Peptides	White fish fillets	peptide hydrolysates of fish fillets	AFC40	Vandanjon et al. (2009)
Antioxidants	Soy protein	Soy protein hydrolysates	G10	Ranamukhaarachchi et al. (2013)
Phenolics	*Ilex paraguariensis* St. Hil. (yerbamate)	Water	HL2521TF	Murakami et al. (2013)

The growing consumer demand for a healthy alternative to sugar has seen the demand for both natural and artificial sweetener products grow dramatically in recent years. Aspartame and sucralose are both widely consumed artificial sweeteners, however consumer demand has seen considerable interest in natural sources of sweetener such as stevia. Stevia (*Stevia rebaudiana)* is a herbaceous plant mainly found in South America and has a sweetness of approximately 300 times greater than sucrose. In addition to the favourable sweetness, stevia has also shown potential for the treatment of type-2 diabetes, obesity, hypertension, inflammation and cancer (Kim et al. 2011). Research on these effects is still in the early stages. The traditional extraction process for the sweet compounds in stevia (stevioside and rebaudioside) involves extraction by organic solvents from the dried leaves, of which the sweetening compounds make up 14% (Zhang et al. 2000). Initial work by Zhang et al. (2000) attempted to reduce the number of unit operations required for the extraction and refinement of the sweetener and minimise the chemical usage. The work undertaken reported water as a suitable extraction medium with extraction temperature a key parameter. pH played no part in sweetener extraction but did affect the quantity of colour components extracted. The authors concluded ceramic microfiltration membranes were suitable for prefiltration and that the use of a nanofiltration membrane adequately concentrated the sweeteners. The application of membranes for stevia purification was revisited in 2011 by Vanneste et al. where a selection of commercial and tailor made polyether-sulphone (PES) membranes were used. The mutli-stage process implemented achieved a product purity of 37% from a feed of 11% using a self-made ultrafiltration membrane prior to the NTR-7450 membrane for concentration. Chhaya et al. (2012) carried out similar work achieving a purity and recovery of 60% while Rao et al. (2012) achieved an extremely high purity of 98.2 %, albeit with a yield of approximately 10%.

2.3.2 Wastewater Treatment (excluding desalination)

Nanofiltration has been successfully applied to the treatment of textile industry waste waters. The textile industry generates vast quantities of highly polluting waste water, with dyeing 1 kg of cotton requiring anywhere from 70 to 150 Litres of

water (Ellouze et al. 2012). A widespread issue in the treatment of textile water is the varying nature of the waste, which is dependent on the product produced and chemicals used. A review of the processing methods in 2004 by Forgacs et al. covered the main technologies used to remove the colour, which include adsorption, photo-catalysis methods, oxidative methods, microbiological and enzymatic decomposition, with no mention of membrane technology and the review concluding that traditional techniques were largely ineffective. Since the publication by Forgacs et al. (2004) the application of membrane technology and in particular NF has grown significantly, although NF had been applied as far back as 1997 (Chen et al. 1997). The research undertaken by Chen et al. (1997) successfully reduced the COD of waste water enabling discharge into a public sewer, whereas future work undertaken by Lopes et al. (2005) successfully reduced the COD while simultaneously removing 99% of the colour allowing for the reuse of the water for dyeing purposes. Similarly, Gozalvez-Zafrilla et al. (2008) successfully used nanofiltration as a replacement for reverse osmosis as a treatment method for water reuse in the textile industry. Mo et al. (2008) applied polyamide NF membranes to five solutions containing five different dye solutions, reporting an almost 100% colour removal producing clear reusable water. The separation when applied to a model feed solution resulted in a high percentage separation but did require pre-treatment using a chemical coagulant prior to application to the membrane and subsequently reporting a 20 % flux increase. A review of the application of polymeric NF membranes to textile dye waste water was carried out by Lau and Ismail (2009). The review covered many aspects of dye filtration from membrane performance, transport modelling and fouling control. However, the authors concluded that the true feasibility of NF for this application is unclear due to the large variability of textile wastewaters as well as the quality of permeates required. However, the cases studied thus far suggest that NF has performed well in highly coloured and highly salinated solutions. Furthermore, a general consensus was that NF offers many advantages when compared to conventional treatment methods. Since the review in 2007, further research into the nanofiltration of textile dyes has been undertaken. Zahrim et al. (2011) reviewed the use of polymeric coagulants as a pre-treatment method for reducing fouling for the

nanofiltration of dyes, reporting an increased efficiency. Ellouze et al. (2012) investigated the use of NF as a post-treatment after coagulation-flocculation (CF), reporting retention figures of 57% for COD, 100% for colour and 30% for salinity. The research was furthered by applying a microfiltration stage as a substitution fro the coagulation-flocculation and reporting a 35 % and 39.6 % increase in COD and salinity retention respectively when compared to a CF pre-treatment. In 2012, Cheng et al. produced a novel self assembled positively charged NF membrane for the treatment of textile industry waste waters, an interesting concept as the majority of NF membranes are negatively charged (Bellona and Drewes, 2005). The membrane produced exhibited a larger fixed charge and a higher flux rate when compared to two commercially available NF membranes, suggesting the membrane is capable of achieving high rejections and high flux rates for low concentrations of cationic species. The membrane when applied to a model low concentration dye solution performed well and achieved a 98% rejection of methylene blue (positively charged dye) at 5 bar. The authors then applied the membrane to a dia-filtration dye desalting process where the concentration of salt was reduced by over 90% in a six hour period allowing for the re-use of the process streams. The further application to an industrially relevant solution and possible comparison with a commercially available negatively charged membrane would have been highly interesting in determining the true potential of positively charged NF membranes in textile water processing. Further membranes were developed by Ong et al. (2014), although in this case the membranes produced were hollow fibre in configuration. The rejection of synthetic dye solutions was studied to assess the performance of the newly constructed membranes, reporting over 90% rejections at most conditions studied, as well as higher than 80% rejection of NaCl. The membranes were studied at both lab and pilot scales and were shown to be robust, showing satisfactory and stable performance under chemical cleaning cycles.

Manttari et al. (2006) studied the potential of nanofiltration for the treatment of wastewater from the pulp and paper industry treated by an external activated sludge process. The results found that NF was suitable for treatment of the wastewater, effectively removing organic compounds and colour traces. However,

the authors noted that should the waste contain high levels of inorganics such as Cl^- ions used to bleach paper pulp nanofiltration may be unsuitable and would require the use of RO.

The past 50 years has seen the development of a wide range of plant protection products, commonly known as 'pesticides', essential for the production of good quality food and fibres. The significant and widespread use of herbicides and insecticides has resulted in their residues being detected in various environmental areas, including surface water and ground water. Frequent detection in natural waters is of great concern to consumers and authorities particularly due to the adverse health effects associated with these compounds even at very small concentrations. The use of nanofiltration for this purpose is hardly new although the topic is seen as an area for development with review papers by Bellona et al. (2004), Bolong et al. (2009) and Plakas and Karabelas (2012) confirming this.

Further examples of waste water treatment were highlighted by Liu et al. (2013) where NF was employed for the removal of melanoidins from molasses fermentation wastewater. The authors used a self made poylamide NF membrane with an approximate MWCO of 580 Da used as a submerged unit. The study focused on optimising the separation performance by assessing colour removal, COD reduction and permeate flux under different trans membrane pressures (TMP) and volume concentration factors (VCF). The results obtained showed melaoidins removal and COD reduction decreasing with increasing TMP and/or VCF at the expense of water permeability. Furthermore, the authors also suggested submerged nanofiltration experiences lower fouling and is easier to clean than direct pressure NF.

2.4 Pharmaceuticals

2.4.1 Pharmaceutical removal from wastewater

Financial pressures in recent years have seen all companies seek to optimise all aspects of production, with the pharmaceutical industry no exception. Traditionally the pharmaceutical industry is the least efficient of all chemical industries in terms of waste generated per unit of product (Rockwell Automation, 2010).

Pharmaceutical compounds are highly valuable and companies strive to maximise recovery of any value from their process streams. However, it is inevitable that some of the active pharmaceutical ingredients (APIs) should be lost to the process waste streams leading to pharmaceutical companies targeting API recovery from such streams. The loss of an active compound not only has a potentially detrimental effect on the process cost efficiency, but also introduces wider environmental and health concerns. A review by Verlicchi et al. (2012) demonstrated the presence of pharmaceuticals from seventeen different therapeutic classes in the effluent stream following treatment by an activated sludge system, the usual reclamation method for urban wastewaters worldwide (Verlicchi et al. 2012). The authors noted both an acute and chronic risk to aquatic life due to the large volumes of waste often discharged. Interestingly there are currently more than 600 biotechnology drug candidates under development in a wide variety of therapeutic areas, suggesting a massive potential for the application of novel separation technologies (Grabowski, 2011).

Attempted recovery of pharmaceuticals have been undertaken using a wide variety of separation technologies such as, oxidation, ultrasonic irradiation, adsorption, oxidation and reduction, membrane filtration and combinatory methods (Homem and Santos, 2011). Studies suggest that membrane processes are suitable for pharmaceutical recovery from waste streams due to the high efficiency, high throughput, ambient operating temperature and no chemical additive requirement of the technology. However, since the pore sizes of micro- and ultra-filtration membranes are greater than that of most pharmaceutical compounds, only nanofiltration and reverse osmosis may be used for the majority of pharmaceutical recoveries (Košutić et al. 2007). Drioli and Curcio (2007) suggested the use of membrane technology as a suitable unit operation in process intensification. Process intensification attempts to improve manufacturing and processing efficiency through reducing equipment size/productivity ratio, energy consumption and waste generation, with membrane separations offering a replacement for conventional energy intensive techniques. An example of process intensification, albeit with ultrafiltration membranes, was carried out by Li et al. (2004) where a 5

kDa UF membrane (tight UF) was used for the pre-treatment of antibiotic solvent extraction feed in order to improve the efficiency and produce a higher quality product. In this case a tight UF membrane performed the desired separation; however the introduction of a NF membrane for concentration may potentially have further increased the efficiency of the extraction or at least reduced the extraction solvent volume required. Dixit and Braeutigam (2007) stressed the importance of prefiltration in pharmaceutical and biotechnology industries. Prefiltration is believed to be vital for the protection of the finer (higher resolution) membranes downstream, subsequently reducing processing time and separation costs. Prefilters are often used to remove precipitates and reduce the bio-burden on the system. Typically the prefilters range from simple filters, to microfiltration/ultrafiltration membranes dependant on the applied process. Although the application of a prefiltration stage is often independent of the final processing technique, their use certainly creates are more efficient NF process. An efficient prefilter should:

- effectively clarify feed to protect downstream unit operations, i.e. remove all solid particulates, reduce range of compounds present.
- maintain a high total throughput.
- have a defined particle retention in order to provide confidence in process safety and efficiency.
- have a low degree of adsorption/adsorption of the target compound.

Pharmaceutical development in the past focused primarily on human biological effect with subsequent environmental impact receiving very little attention. "In recent years, pharmaceutical compounds (PhCs) have provoked increasing concern, particularly as no legal requirements have been set for discharge into surface water bodies of these ubiquitous, persistent and biologically active substances" (Verlicchi et al. 2012). The persistent discharge of these compounds are bound to cause cumulative detrimental effects as these compounds are produced with the intention of performing a biological effect (Halling-Sorensen et al. 1998). Furthermore, traditional water treatment plants are not specifically designed to remove pharmaceutical active compounds, hence the potential of NF (Vergili,

2013). Košutić et al. (2007) et al. investigated the removal of eight veterinary antibiotic pharmaceuticals from model waste water using NF and RO technology. The study found that both the RO and tight NF membranes rejected an acceptably high quantity of the antibiotics tested, with the tight NF membrane rejecting 99.1 % of the smallest API tested (Sulfaguanidine, MW: 214.2). However, the looser NF membrane (Desal HL) rejected 67.3% of the same compound, thus suggesting membrane selection plays a vital role in successful API recovery. The relationship between membrane porosity and solute rejections was examined and the results obtained suggested that the dominant rejection mechanism of the unionazable antibiotics was size exclusion. The authors concluded that when flux is considered the NF90 membrane displayed optimal performance, demonstrating comparable rejections to the RO membranes with a higher flux. Radjenovic et al. (2008) studied the rejection of pharmaceuticals from waste water using NF membranes on a large scale. The study concluded that the highest rejections were observed for the negatively charged pharmaceuticals as would be expected due to the negative nature of most NF membranes. However, the results were variable as some of the negatively charged pharmaceuticals present were poorly rejected (<70%). Overall the NF and RO membranes studied performed efficiently removing nearly all the pharmaceutical residues detected. The removal of trace contaminants from a pharmaceutical waste water stream using a custom made NF membrane prepared by phase inversion was studied by Swamy et al. (2013). The study highlighted that despite slightly lower rejections observed with NF the economic estimation of the process make NF favourable compared to RO. Wei et al. (2010) furthered the understanding of nanofiltration by investigating membrane fouling associated with the processing of pharmaceutical wastewater. The study highlighted the relative scarcity of fouling investigations using real process wastewater, therefore the authors used raw biotreatment wastewater effluent from a sequencing batch reactor pre-treated by a UF membrane. The investigation concluded that fouling was due to a mixture of organic and in-organic foulants and that there were two distinct fouling stages. The initial stage was mostly the deposition of sulphate and carbonate of calcium due to the high concentration in the feeds and concentration polarisation. The second fouling stage was the deposition of organic matter, partly

due to the reduction in membrane charge caused by the initial fouling stage. The authors summarised that a cleaning process requires a cleaning agent able to efficiently break down the compact foulant layers by reacting with the species. In the case studied by Wei et al. (2010) a flux recovery of 99% was observed after cleaning. The effect of membrane fouling by emerging organic contaminants was researched by Xu et al. (2006) for a variety of NF and RO membranes. The findings found that fouling significantly affects the rejection of organic solutes by NF membranes, however due the extremely complex fouling process more research is required to understand the membrane-foulants-solutes interactions. Understanding these interactions would provide further insight into the solute exclusion mechanisms. Vergili (2013) studied the rejection of low-molecular weight (<300) pharmaceutically active compounds from wastewater using a membrane with a 1000 MWCO, and observed low rejections (<61%) concluding the separation was charged based. Vergili (2013) used Fourier Transform Infrared Spectroscopy (FT-IR) to study membrane fouling and similar to Wei et al. (2010) reported the deposition of calcium salts on the membrane surface. Removal of pharmaceutically active compounds from waste waters is well researched with all reporting acceptable NF separation performance (Yoon et al. (2006); Yoon et al. (2007); Yangali-Quintanilla et al. (2009); Zazouli et al. (2009); Alturki et al. (2010); Acero et al. (2010); Ravikumar et al. (2014)). However, the effect of fouling on antibiotic removal cannot be ignored with Lin et al. (2014) highlighting that silica fouling on NF membranes may lead to an increase or decrease in pharmaceutical removal from waste water dependant on membrane MWCO. The work by Lin et al. (2014) reported when a membrane MWCO is low (100) the build up of silica promoted higher pharmaceutical rejection from waste water due to the size exclusion being the dominant mechanism. When a membrane with MWCO of 300 is used electrostatic repulsion dominated the separation. Despite the development of a silica foulant layer increasing the steric rejection properties, the hydrophilic deposition results in greater impedance of the membrane back diffusion which therefore increased the diffusion of the pharmaceutical compounds and decreased rejection. Urase and Sato (2007) investigated the change in pharmaceutical retention when a nanofiltration membrane deteriorates. The work undertaken

showed that when a NF membrane is exposed to chlorine both the electrical expulsion and size based rejection mechanisms are affected. Overall, Urase and Sato (2007) highlighted the need to constantly monitor the separation efficiency as the study showed lower retention in an acidic environments attributed to an increase in pore size after chlorine exposure.

Further studies utilising NF for antibiotic removal have seen Homayoonfal and Mehrnia (2014) develop a custom nanofiltration membrane by the phase inversion of an ultrafiltration membrane for the removal of amoxicillin. Martinez et al. (2013) studied the use of nanofiltration membranes for the improved recovery of 1-(5-bromo-fur-2-il)-2-bromo-2-nitroethane (referred to as G1), a pharmaceutically active ingredient used as a precursor to many pharmaceutical products. The purification of G1 typically loses 250g of product per batch through the use of 9.4 litres of ethanol as a washing agent. Previous work by Martinez et al (2012) recovered over 80% of G1 in a synthetic G1/ethanol solution; however the authors believed the presence of other compounds may affect the performance and recovery. The research undertaken into the purification of a realistic solution found that a recovery of 60% was possible in a single stage using the DuraMem 150 and NF 90 membranes despite the presence of pyridine; however, when the concentration of pyridine increases the retention and permeability of G1 decreases. The authors concluded the need for a multi-stage process to achieve optimum separation and purification and that a purity of 80% G1 was economically viable using NF technology.

2.4.2 Pharmaceutical recovery

The organic compound p-anisic acid, derived from anise, has been shown to have uses in the treatment of Parkinson's disease, hepatitis B and C viruses and liver disease (Gandhi and Murthy, 2013). The research undertaken by Gandhi and Murthy (2013) used two membranes, one commercially available membrane and a goat skin parchment membrane reported to be suitable for nano range experimentation. The work undertaken attempted to further the knowledge of the

penetration of chemicals, drugs and other similar pharmaceutical substances through dermal and other biological barriers.

The growing role of NF in the production of biopharmaceuticals was highlighted by Morao et al. (2006) who successfully utilised NF technology for the concentration of clavulanic acid from a clarified fermentation broth. The impact of operating in a biological system was highlighted by the authors who noticed a significant flux decline due to concentration polarisation with enhanced osmotic pressures. The theoretical separation of clavulanic acid was subsequently modelled by Morao et al. (2008) using the Nernst-Planck equations and the steric, electric, dielectric and exclusion model, with the results obtained successfully predicting the rejections of the five studied ions. Membrane technology was also applied to fermentation broth concentration by He et al. (2009), where a combined UF-NF process was applied for the filtration and concentration of the fermentation broth of the antibiotic erythromycin. The work undertaken showed the advantages of a combined membrane process in improving downstream efficiency and reducing the consumption of extracting solvent required with the added benefit of using the NF permeate as the UF diafiltration water. A specialist application of NF was studied by Menconi et al. (2009), where NF was used for the removal of non-enveloped viruses from three plasma-derived products. The study focused on the removal of parvovirus B19 and torque teno virus from three plasma derived products prothombin complex (PTC), albumin solution and factor IX. The results published showed a variation in the rejection of the viruses in the three solutions studied, however the authors summarised that nanofiltration can be efficacious in removing small viruses, although large-scale constant process monitoring would be required. Further work regarding the removal of viruses from plasma derived products was undertaken by Caballero et al. (2014), where a wide range of viruses (including HIV, West Nile virus, pseudorabies virus) were shown to be adequately removed for the blood plasma products tested. The results obtained by Caballero et al. (2014) were in agreement with Menconi et al. (2009), highlighting the consistent robustness of nanofiltration for virus removal. Low et al. (2007) discusses the need for efficient cleaning materials in order to help lengthen the life of nanofilters used for virus

removal. Furthermore NF has been shown to be partially successful for the removal of prions with Yunoki et al. (2008) reporting a 15 nm filtration removing prions to below a detectable limit. The authors reported however than re-infection may reoccur post nanofiltration.

2.5 Membrane fouling

Membrane processes are of great interest because they reduce the number of unit operations, recycle process water and recover valuable compounds. Furthermore they are considered an attractive alternative to conventional wastewater treatment methods due to their inherent advantages such as selective separations, continuous and automatic operation, no additional chemical requirement, ease of scale up and low space requirement (Guo et al., 2012). Despite the many advantages of membrane technology there are a number of factors which determine the success of a membrane process, these include initial capital cost, power requirements, membrane life-span and replacement costs. One of the major issues impacting the success of any membrane process is the prevention of flux decline by the prevention or reduction of fouling, scale inhibition and the implementation of successful cleaning protocols. The principles and causes of nanofiltration fouling are covered in great depth by both Schäfer et al., (2004) and Al-Amoudi and Lovitt, (2007).

Membrane fouling is a major impediment in the widespread application of membrane technology (Vogel et al., 2010). The development of fouling control strategies has been a major challenge in membrane research. However, despite the development of many preventative strategies, fouling is generally considered as an inevitable feature of the membrane process (Ang et al., 2006). The term fouling is used to describe the undesirable formation of deposits on the membrane surface, a phenomena that occurs when the rejected particles are not transported back into the bulk feed solution. In order to effectively reduce membrane fouling, or to clean a fouled membrane, the nature and properties of the fouling material must be identified. Foulants are typically colloidal materials of one sort or another and the properties and interaction these foulants with the membrane surface governs the fouling/cleaning process. Colloids are defined as suspended particles in the size range of a few nanometres to a few micrometres (Al-Amoudi and Lovitt, 2007). The fouling materials are normally classified as inorganic (clays, silica, metal oxides, mineral scale), organic (Natural organic material such as proteins and carbohydrate, anti-scalants) or biological (bacteria, algae, fungi and other micro-organisms), however the

specific fouling observed in any process may be a combination of all three (Tang et al., 2011).

Fouling of a membrane can be divided into surface fouling (build-up of a cake/gel-like layer on the membrane surface) and pore blocking (build-up of foulants at the pore entrance either plugging the pore or bridging over pores). In the case of frontal (dead-end) filtration pore blocking may be further divided into three types: complete pore blocking (blocking of the membrane pore by a particle of similar size), incomplete membrane fouling (known as intermediate fouling) and standard pore blocking (gradual pore narrowing and constriction by a particle much smaller than the pore size) (Al-Amoudi and Lovitt, 2007). Guo et al., (2012) identified six principal fouling mechanisms:

1. Pore blocking
2. Cake Formation
3. Concentration Polarisation
4. Organic Adsorption
5. Inorganic Precipitation
6. Biological Fouling

The flux decline of a NF membrane is normally attributed to pore blocking and is observed in both dead-end and cross-flow systems. Flux decline due to fouling leads to an increase in production costs due to an increase in energy demand, requirement for chemical cleaning materials, downtime for cleaning in place, reduction in membrane life expectancy and the additional labour required for maintenance.

The nature of a membrane process where a feed stream is concentrated can lead to significant inorganic fouling in the form of scale formation. The concentration of a feed stream can lead to inorganic species reaching their solubility limits which results in the precipitation of the material on to the membrane surface. In order to prevent the formation of scale it is necessary to understand the chemistry of the feed stream and either operate the system below the solubility limit or adjust the system chemistry accordingly to prevent the precipitation. The development of membrane scaling by compounds such as calcium carbonate, calcium sulphate and silica results in a significant flux decline and can lead to membrane damage (Schäfer et al., 2005). The crystallisation process is influenced by a number of factors within a membrane system including pH, temperature, flow velocity, permeation rate, membrane properties, salt concentration, and concentration

polarisation. These several factors either when considered alone or combined play a vital role in crystallisation and subsequent cake formation (Al-Amoudi and Lovitt, 2007). The issue of inorganic fouling is further complicated by the issue of mixed feed solutions which alters the chemical properties of the solutes compared to being in a pure state.

Nanofiltration is perceived as an alternative for the treatment of water that contains natural organic matter (NOM) (Mattaraj et al., 2011) The flux decline observed through the filtration of natural organic matter may be classified as reversible or irreversible (Guo et al., 2012). Reversible flux decline may be fully restored by the undertaking of a suitable chemical cleaning step. However, irreversible flux decline due to NOM may not be removed even when subjected to a rigorous chemical clean. Membrane fouling by NOM is influenced by: membrane characteristics, pH, concentrations of mono- and di-valent ions present, the properties of the NOM including polarity and molecular weight, system hydrodynamics, applied pressure, mass transfer properties and concentration polarisation (Al-Amoudi and Lovitt, 2007).

Membrane systems are prone to fouling by biologically active organisms, a phenomena commonly known as biofouling. Membrane biofouling is caused by bacteria, and to a lesser extent fungi, algae and other eukaryote microoragnisms adhering to the membrane surface (Wibisono et al., 2015). The use of elevated temperatures (in the region of 40°C) is often used in industrial membrane processing due to the increased flux obtained, however, this provides an ideal growth temperature for many microorganisms leading to the formation of biofouling. The formation of biofouling is a dynamic process of microbial colonisation and growth which results in a microbial biofilm on the membrane surface. Biofilm formation precedes biofouling which becomes an issue once the biofilm reaches a significant thickness to cause problems such as flux decline or increased pressure drops across membrane modules. The microorganisms living in the biofilm are normally surrounded by excreted extracellular polymeric substances (mainly polysacchardies, proteins, nucleic acids and lipids) which further reduce the flux. The extent of biofouling may be controlled through removal of degradable components (feed materials for the biofilm) from the feed water, ensuring that the introduction of degradable components is kept to a minimum and that efficient cleaning procedures are undertaken (Al-Amoudi and Lovitt, 2007; Alzahrani et al., 2013).

2.5.1 Membrane Cleaning

The cleaning of fouled nanofiltration membranes is a very active area of membrane research, with a number of authors investigating cleaning methods such as two-phase cleaning (Wibisono et al., 2015), membrane backflushing (Jiang et al., 2015) and chemical cleaning (Simon et al., 2013a). Membrane flux remediation is normally undertaken by chemical cleaning procedures. The frequency of cleaning required is highly dependent on the industrial sector, for example whey processing requires daily cleaning while the a desalination plant requires much less frequent cleaning (often only annual cleaning is required). The implementation of a suitable pre-treatment stage is essential for the reduction of fouling experienced by nanofiltration membranes, however, this will not entirely prevent fouling occurring over time. A further advantage of a suitable pre-treatment stage is the possible reduction of the membrane cleaning frequency required (Guo et al., 2012). Another method of fouling prevention is membrane surface modification in order to reduce the adhesive potential of organisms (Tang et al., 2011). This prevention method is currently tailored to specific systems which would be prohibitively expensive on an industrial scale.

Membrane cleaning methods may be separated into four types, namely: physical, chemical, physio-chemical and biological methods. The method predominately used is chemical cleaning. The complexity and detailed understanding of cleaning processes has not yet been addressed by researchers and is an area that needs clarification for a clear knowledge of these operations. (Al-Amoudi and Lovitt, 2007).

Physical cleaning methods often attempt to 'mechanically' remove foulant materials from the membrane surface. Physical cleaning methods include backflushing, where the permeate side of the membrane is pressurised which pushes the permeate back through the membrane (Zhao et al., 2000; Guo et al., 2012). This process aims to dislodge the foulant material and is highly dependent on membrane suitability for back flushing. A method of cleaning often used for tubular membranes is that of sponge balls (Shi et al., 2014), where a polymeric ball is placed within the membrane tube to 'scrub' the foulants away from the membrane surface. The use of sponge balls is limited as they cannot be used on spiral wound membrane modules. Further physical methods include ultrasonic vibration and air sparging in order to remove foulants.

Chemical cleaning is the most widely used membrane remediation technique. A large range of chemical cleaning agents are commercially available and these chemicals normally fall into six categories: alkalis, acids, metal chelating agents, surfactants, oxidising agents and enzymes. Commercially available materials are normally a mixture of these compounds although the true mixture is rarely known (Al- Amoudi and Lovitt, 2007). The success of a membrane cleaning is dependent on several factors, these include pH, temperature, chemical concentration, cross-flow velocity (hydrodynamic shear), cleaning duration, and cleaning pressure.

Biological cleaning methods use bioactive agents in order to regenerate the membrane surface (Schäfer et al., 2004). The use of enzymes presents an ideal cleaning agent as they are able to specifically interact with specific foulant. Furthermore enzymatic agents are often environmentally friendly and operate at neutral pH and temperature, therefore do not damage the membrane surface.

2.5.2 Concentration Polarisation

One reoccurring theme in membrane fouling is that of concentration polarisation. Concentration polarisation (CP) is defined as the accumulation of solute species on the upstream (feed) side of the membrane surface. Concentration polarisation is considered to be a hydrodynamic/diffusion phenomenon. CP appears to some degree in every membrane system because of the fundamental limitations of mass transfer and the existence of a boundary layer, however the effects may be suppressed through operating the system at a higher velocity or by efficient stirring in frontal filtration mode (Zhao et al., 2000).

Figure 2.1 shows a schematic diagram of the interface between the bulk feed solution and the membrane surface for a single electrolyte, i.e. the concentration polarisation layer.

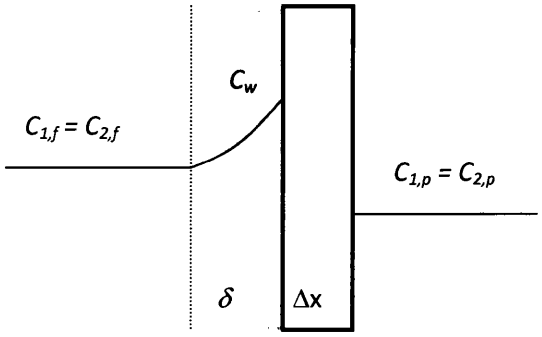

Figure 2.1: Concentration profiles within the polarised boundary layer.

The concentration near the membrane surface (C_w) is higher than that of the bulk concentration (C_1). The solutes are drawn towards the membrane surface via convection and diffuse back in to the bulk solution in a balanced steady-state concentration gradient. CP differs from fouling in that fouling deposits material on the membrane surface whereas CP maintains a higher solute concentration near the membrane surface. The two phenomena are closely related. Concentration polarisation may affect membrane performance in a number of ways (Tang et al., 2011):

1. The development of a significant CP layer near the membrane surface can cause the osmotic pressure to be significantly higher than the bulk solution resulting in decreased membrane flux.
2. When there is a porous foulant layer present on the membrane surface the rate of back diffusion from the surface may be reduced resulting in a significant increase in the CP layer and hence a flux decrease.
3. The presence of a CP layer results in an increased concentration of fouling solutes near the membrane surface than that of the bulk solution. The system when operated at a high membrane flux may result in the deposition of a solid phase (particle deposition/gel layer formation). Increased colloidal concentration at the membrane surface is also known to increase the rate at which fouling occurs.

2.6 Relevant Membrane Theory

2.6.1 Calculation of Rejection

The experimental observed rejection from a membrane surface is defined as (Mohammad et al. 2007):

$$R_{obs} = 1 - \frac{C_p}{C_f} \qquad [2.1]$$

where C_p and C_f are the concentrations of the permeate and feed respectively. In reality, due to concentration polarisation this definition of rejection is not accurate because the concentration at the membrane surface, C_w is higher than the bulk feed concentration; C_f. The real rejection of the solute, R_{real}, which is always equal to or higher than R_{obs} is defined as:

$$R_{real} = 1 - \frac{C_p}{C_w} \quad [2.2]$$

The concentration at the wall, C_w, cannot be measured directly and therefore must be calculated indirectly with a suitable model for concentration polarisation (Nicolas et al. 2000). The approach to concentration polarisation taken in this study is that of the infinite rejection method described previously (Nakao and Kimura, 1981; Opong and Zydney, 1991):

$$\exp\left(\frac{J_v}{k}\right) = \frac{C_w - C_p}{C_f - C_p} \quad [2.3]$$

where J_v is volumetric flux through the membrane and k is the mass transfer coefficient in the polarised boundary layer, defined as:

$$\mathrm{k} = \frac{D_{eff,\infty}}{\delta} \quad [2.4]$$

and $D_{eff,\infty}$ is the effective diffusion coefficient at infinite dilution and δ is the thickness of the concentration polarisation layer. This result is equally applicable to a system of uncharged solutes. The exponential term represents the mass transfer effect and shows that when $C_w = C_f$, the exponential becomes unity, and when concentration polarisation is occurring the exponential becomes greater than unity. The mass transfer coefficient may be determined experimentally by linearization of Eq. [2.3], yielding

$$\ln\left(\frac{1 - R_{obs}}{R_{obs}}\right) = \frac{J_v}{k} + \ln\left(\frac{1 - R_{real}}{R_{real}}\right) \quad [2.5]$$

In this case, the mass transfer coefficient is expressed as

$$k = a\omega^n \quad [2.6]$$

where a and n are constants and ω is the membrane cell stirrer speed.

This result is applicable for uncharged solutes, but the correlation for a multicomponent electrolyte system requires the solution of the extended Nernst-Planck equation (Bowen and Mohammad, 1998). The exponential term in equation [2.3] is known as the polarisation modulus and is a dimensionless term representing the extent of mass transfer and shows that when $C_w = C_f$ the modulus becomes unity and when concentration polarisation is occurring the modulus increases in magnitude. Thus, the polarisation modulus offers a simplistic quantitative comparator of mass transfer effects in different equipment. Many mass transfer correlations have been derived to predict k for simple membrane modules such as tubular and hollow-fibre membranes (Rautenbach and Albrecht, 1994), frontal filtration equipment (Opong and Zydney, 1991) and various other membrane geometries (Nguyen et al. 1980; De and Bhattacharya, 1997; Minnikanti et al. 1999; Geraldes and Afonso, 2006). These correlations relate the dimensionless Sherwood number to Reynolds number and Schmidt number, i.e.:

$$N_{Sh} = \frac{kr}{D_{eff,\infty}} = \varphi(N_{\mathrm{Re}})^{n}(N_{Sc})^{0.33} \qquad [2.7]$$

where

$$N_{\mathrm{Re}} = \frac{\omega r^2}{\upsilon},\ N_{Sc} = \frac{\upsilon}{D_{eff,\infty}} \qquad [2.8]$$

For example the most suitable value for the empirical constant n was found to be 0.567 (Malone and Anderson, 1977; Opong and Zydney, 1991) in the Amicon cell. Bowen and Mukhtar (1996) and Bowen et al. (1997) used this correlation to account for concentration polarisation in the characterisation of nanofiltration membranes, namely

$$k = 0.23\left(\frac{r^2}{\upsilon}\right)^{0.567}\left(\frac{\upsilon}{D_{eff,\infty}}\right)^{0.33}\frac{D_{eff,\infty}}{r}\omega^{0.567} \qquad [2.9]$$

The estimation of mass transfer using accurate correlations allows accurate prediction of membrane separation performance, however, van der Berg et al. (1989) highlighted the pitfalls in blindly using correlations and recommended the evaluation of the exponent n from experimental data. Experimentally, the real rejection may be determined from the observed rejection on extrapolation to infinite stirrer speed by plotting $\ln [(1 - R_{obs})/R_{obs}]$ against J_v/ ω^n. The slope of the best fit line will be equal to 1/a. The real rejection may then be calculated by rearranging Eq. [2.5] (Oatley et al, 2012) to yield:

$$R_{real} = \frac{1}{\exp\left(\ln\left[\frac{1-R_{obs}}{R_{obs}}\right] - \frac{J_v}{a\omega^n}\right)+1} \qquad [2.10]$$

The value of *R* obtained is the real rejection at infinite stirrer speed.

2.7 Organic Solvent Nanofiltration (OSN)

The dramatic rise of NF technology in the past 25 years has seen the application of NF to many industrial separations. A safe assumption is that the maximum potential of NF has not yet been reached. Despite the many advantages of NF, as with any new technology NF does have some disadvantages such as membrane fouling and insufficient purification, issues that were thoroughly reviewed by Van der Bruggen et al. (2008). Membrane fouling and membrane cleaning in itself has become an area of great research (Al-Amoudi & Lovitt 2007). The majority of NF applications thus far have been in the aqueous phase mainly due to shortcomings in the materials of manufacture; polymeric membranes upon contact with organic solvents tend to swell and reduce their separation capabilities (Machado et al. 1999). The future success of NF technology will inevitably require the use of the technology for separations in an organic solvent environment. The ability to undertake selective separations with solvent-containing solutions would enable the recycling of solvents, the concentration and purification of solvents, and the recovery of valuable materials (Schafer et al. 2005), particularly when considering the majority of pharmaceuticals are produced in organic solvent environments and account for 80-90% of the mass in a pharmaceutical process (Constable et al. 2007;

Grodowska and Parczewski, 2010). The majority of solvents used in the processing are rarely recycled, therefore the application for solvent recovery alone has massive potential (Perez-Vega et al. 2011).

Two main terms have been used to describe the process, namely 'Organic Solvent Nanofiltration' (OSN) and 'Solvent resistant nanofiltration' (SRNF) (Volkov et al. 2008). OSN is now the predominantly used term. Nanofiltration involving organic solvents is currently a niche sector of membrane separations, although with improved technology, particularly in membrane stability, increasing applications in petrochemicals, pharmaceuticals, fine chemicals and some large scale processes should be seen. OSN membranes were developed through the process of applying NF membranes to non-aqueous solvent separations. These non- aqueous solvents included hexane, methanol, ethanol, ketones, aldehydes, ethyl acetate, acetonitrile, tetrahydrofuran and dimethyl formamide, toluene, DMSO, which are largely encountered in the petrochemical, biotechnology, pharmaceutical, and chemical industries, albeit with varying success (Razdan & Joshi 2003). The current development of OSN was thoroughly reviewed by Vandezande et al. (2008) where membrane materials, applications and modelling of OSN were discussed.

The future success of OSN will depend predominantly on the development of suitable membrane materials to survive and perform adequate separation in the harsh operating environment of organic solvents. OSN membranes similar to aqueous systems fall into two distinct categories: 1) integrally skinned asymmetric membranes and 2) thin film composite (TFC) membranes (Peyravi et al. 2012). recent years has seen research into the generation of novel membranes for solvent application as well as surface modification of existing NF membranes. Surface modification, chemistry and morphology are key to an efficient membrane process, and modification of polymers potentially allows the production of custom membranes, which can be tailored to be process dependent (Aerts et al. 2006; Seman et al. 2010). The number of commercially available OSN membranes is limited with only a few companies currently producing OSN membranes worldwide (Peeva et al. 2010).

Current applications of aqueous phase nanofiltration are wide and varied, whereas the lack of suitable OSN membranes has hampered the growth of the technology in the non-aqueous phase particularly when compared to aqueous applications. The application of OSN is growing rapidly with applications such as stream recovery in the dyeing industry, separation of active pharmaceutical ingredients (API's), catalyst recovery and regeneration, solvent recovery, separation of oils and emulsions, recovery of free fatty acids from vegetable oils and solvent recovery of oils (Kosaraju & Sirkar 2008; White & Nitsch 2000; Scarpello et al. 2002; Han et al. 2005; Whu et al. 2000; Sheth et al. 2003; Nwuha 2000; Pagliero et al. 2007; Coutinho et al. 2009; Zwijnenberg et al. 1999; Geens et al. 2007). The application of organic solvent nanofiltration to the extraction of bioactives is increasing year on year, mainly as the majority of biological research relies on their use as extraction media. Tsibranska and Saykova (2013) reviewed the use of OSN for the concentration and fractionation of polyphenols from plant extracts in combination with other technologies (crystallisation, precipitation, adsorption). The review concluded that the use of OSN in combination with other techniques extends the potential of membrane technology for the isolation of biologically-active compounds from natural extracts. Table 2.2 below outlines selected applications of OSN over a variety of sectors.

Table 2.2: Applications of OSN

Application	Membrane	Solvent	References
Solvent Recovery			
Solvent recovery from API crystallisation	StarMem 122, 240, 280	Isopropyl acetate	Rundquist et al. (2012)
Ionic liquids Separation	StarMem 120, StarMem 122, StarMem 280, StarMem 240	Methanol, toluene, ethyl acetate	Han et al. (2005)
Co-Jacobsen Catalyst Recovery	Desal DL, Desal GE, TFC-SR2, MPF-50, COK M2, PDMS, NF-PES-10, N30F, P005F, MPF-44, PERVAP	Di-ethyl ether, Isopropanol	Aerts et al. (2004)
Catalyst Recovery (Tetraoctylammonium bromide)	142A, 142C	Toluene	Luthra et al. (2001)
Catalyst Recovery (Grubbs type catalyst – Gr1, Gr2, HGr1, HGr2)	StarMem 120, StarMem 122, StarMem 228, StarMem 240	1-Octene, toluene	Van der Gryp et al. (2010)
Catalyst Recovery (Quinine Based Catalysts – BzCPN)	DuraMem 150, DuraMem 200, DuraMem 300, DuraMem 500	Tetrahydrofuran	Fahrenwaldt et al. (2013)
Organometallic catalysts (Jacobsen, Wilkinson, Pd-BINAP catalysts)	MPF-50, STARMEM 122, STARMEM 120, STARMEM 240 Desal-5	Ethyl acetate, Tetrahydrofuran, Dichloromethane	Scarpello et al. (2002)

Solvent exchange	MPF-50, MPF-60	Methanol, Ethyl Acetate	Sheth et al. (2003)
Pharmaceutical			
API recovery	StarMem 120, 122, 228	Toluene, methylene chloride, methanol	Geens et al. (2007)
API recovery (1-(5-Bromo-fur-2-il)-2-bromo-2-nitroethane)	PES/NMP 25%, PES/NMP 27%, PES/NMP 30%, PES/NMP 32%, NF 30F, NF 270, NF 90, Desal 51 HL	Ethanol	Martinez et al. (2012)
API recovery and purification	DuraMem 150, 200, 300, 500	Tetrahydrofuran, Dimethylformamide	Sereewatthanawut et al. (2010)
Peptide recovery	SelRO MPF-50, DuraMem 1000	Dimethylformamide, dichloromethane, di-ethyl ether, ethanol	So et al. (2010)
API Purification (Genotoxic impurity removal)	GMT-oNF-2, SolSep NF010206, MPF-44	Methyl ethyl ketone, Tetrahydrofuran, dichloromethane	Szekely et al. (2011); Szekely et al. (2012)
API Purification (Genotoxic impurity removal)	DuraMem 150, Custom PBI membranes	Methanol	Peeva et al. (2014)
Organic intermediates (safranin O, brilliant blue R, vitamin B_{12})	MPF-44, MPF-50, MPF-60	Methanol	Whu et al. (2000)
Emulsions			
Metalworking fluid (MWF) treatment	AFC 30	Mobilcut 232 (Semi-synthetic MWF)	Hilal et al. (2004)

(Mobilcut 232)			
Solvent dewaxing (lube oil)	Polyimide (Lab made) (6FDA polymer)	Methyl ethyl ketone (MEK), toluene	Kong et al. (2006)
Solvent dewaxing (lube oil) Preceded MAX-DEWAX®	Polyimide (Lab made) Matrimid® 5218 (Ciba-Geigy Corp.)	Methyl ethyl ketone (MEK), toluene	White and Nitsch, (2000)
Biodiesel separation	Solsep 030705, Solsep 030306F, StarMem 240, StarMem 120, Desal-DL, Desal-DK, MPF-34, MPF-44	Biodiesel, glycerine, methanol,mono/di/tri - glycerides	Othman et al. (2010)
Oily Wastewater	NE2540-70, NE2540-90 (Commercial), Two Polyamide on polysulfone support (Lab made)	Oily wastewater	Rahimpour et al. (2011)
Dye Separation	X-20 hollow fibre membranes (Lab Made)	Methanol	Kosaraju & Sirkar 2008
Food			
Antioxidant extract concentration (rosemary)	DuraMem 200, DuraMem 300, DuraMem 500	Ethanol	Peshev et al. (2011)
Ethanolic extracts (*Sideritis ssp.* L.) ironwort	DuraMem 300, DuraMem 500	Ethanol	Tsibranska & Tylkowski (2013)
Green tea	HC-50, HR98PP	Ethanol	Nwuha (2000)
Degumming -	PVDF membrane	Water,	Pagliero et al. (2007)

Sunflower and soybean oil	(Lab made)	Hexane	

(PES – Polyethersulfone, NMP – N-methyl-pyrrolidone, PDMS – Polydimethylsiloxane, COK M2 – Silicon based membrane with inorganic filler, PBI - Polybenzimidazole, PVDF - Polyvinylidenfluoride), (142A, 142C – Both W.R.Grace, USA; N30F, P005F and NFPES10 - All Nadir; TFC-SR2 – Koch membrane; PERVAP – Sulzer; AFC 30 – PCI Systems, NE2540-70, NE2540-90 – SAEHAN Corp., Korea; DuraMem – Evonik Industries; SelRO MPF – Koch membranes;, SolSep – SolSep BV, GMT-oNF-2 – Borsig Membrane Technology GmbH)

OSN is a separation technique with great potential, both currently and undoubtedly in the future. The development of OSN technology has come a long way since inception approximately 15 years ago, although there is a great deal or development required prior to OSN being a feasible and truly exploitable process for the world's future separation needs. Despite the many varied applications of OSN the largest issue facing the sector is long term membrane stability. The majority of the literature works cited show successful performance for the desired separation but often only conduct the experimentation over a short period and therefore offer no information for the true long term performance should the process be industrially implemented. The continued development of OSN membranes will eventually offer membranologists another tool in their separation arsenal and will surely see the growth of nanofiltration in general.

2.8 Concluding remarks

The application of NF to biological applications is wide and varied; however NF has yet to reach the technology's maximum potential. The majority of this review concerns the application of NF to aqueous environments. Many of the applications reported concern the treatment of water and in particularly the removal of biological 'wastes' from water. Clean water is a scarce commodity in many parts of the world, making the recycling and purity of water extremely important. The true potential of NF would be further realised by application in the production of high value bioactive compounds such as pharmaceuticals. Such examples have been reported in this review, however with development in membrane technology ever

increasing, NF technology will inevitable play a prominent role in future separations and concentrations of biologically active compounds.

Nanofiltration like many new technologies is faced with the issue that individuals and organisations are reluctant to accept significant changes. The application of nanofiltration to water treatment has been widely accepted and exploited, mainly due to the fact that chemical engineers were initially tasked with water desalination using evaporation etc; the development of nanofiltration therefore led to eager engineers readily accepting the new technology. Nanofiltration for biological applications is less widely accepted, however this review has highlighted some of the major inroads being made by the technology. The boundaries present between engineers and scientists, and the often inherent difference in scale of operation, is often responsible for the slow acceptance and implementation of the technology. Greater collaboration should see increased implementation of nanofiltration and the associated benefits that the technology provides. This literature review also highlights a lack of applications for nanofiltration technology to biological separations where the materials of interest have potential bioactivity. Tsibranska et al. (2011) and Tylkowski et al. (2011) both studied the use of membrane technology and solvent extraction for the separation of polyphenols and flavonoids from *Sideritis* ssp. L. plant. The studies showed that the antioxidant activity increased through the use of a membrane based process for the concentration and purification process. The literature reviewed has revealed very few other examples where a membrane process has been applied to a biological separation with the goal of maintaining product bioactivity. For this reason, this thesis aims to apply membrane technology to the novel application of separating and purifying extracellular bioactives from a fungal source while ensuring the maintenance of bioactivity. Furthermore, membrane technology will be applied to a novel separation process for the extraction of a small bioactive from waste potato peel on both a laboratory and pilot scale. Both of these separations will demonstrate the nanofiltration process and highlight that the technology can deliver real benefits to biological separations.

3.0 Materials and Methods

This chapter details the general materials and methods used in the experimental work, specific details of experimental materials and methods are included at the start of each relevant chapter. The experimental work performed during the study includes: rejection experiments of salt solutions and uncharged solutes, membrane characterisation by means of pore size and zeta potential charge measurements and determination of equipment mass transfer characteristics.

The experimental investigation of the nanofiltration theory as well as the majority of the scoping work for the biological separations were undertaken in laboratory scale frontal (dead-end) filtration cells and therefore will be described in detail. Frontal filtration, while different in operating principle to industrial cross-flow systems allows accurate process prediction on an industrial scale once the frontal filtration equipment properties are understood. A disadvantage of frontal filtration systems is that the membranes are prone to fouling and therefore must be replaced frequently. However, the very nature of experimental work results in membranes rarely being re-used on a laboratory scale. The general materials and techniques used for experimental work will be provided. Finally, supporting experiments will be presented to investigate the mass transfer characteristics of the dead-end filtration cell and the experimental characterisation of a nanofiltration membrane used throughout the study.

3.1 Membrane Experimental Equipment

3.1.1 Laboratory scale membrane equipment

Rejection experiments are of paramount importance for the understanding of a membrane process. Therefore the development of an experiment that allows the accurate testing of membranes is vital. The experimental work undertaken at the laboratory scale used three commercially available stirred frontal filtration membrane cells, namely the Amicon 8050 (Merck Millipore UK Ltd. Watford, UK), the Sterlitech HP4750 (Sterlitech Corporation, Washington, USA) and the Membranology HP350 (Membranology Ltd., Swansea, UK). The use of three

different commercial cells allowed the mass transfer characteristics of each piece of equipment to be studied. The properties of the three cells are shown in Table 3.1:

Table 3.1: Properties of the three frontal filtration cells used

Property	Amicon	Sterlitech	Membranology
Membrane Area (cm^2)	13.4	14.6	41.8
Operating Volume (mL)	50	300	350
Maximum Operating Pressure (bar)	5.17	69	100

Schematic diagrams of the Amicon 8050 stirred cell and the Membranology cell are shown in Figure 3.1 and 3.2 respectively. The Amicon cell consists of a plastic cylindrical body, a membrane support, small channels to allow permeate to flow out, a quick fit base (which holds the membrane support and body), a magnetic stirrer assembly which is mounted inside the body, a plastic top cap containing a pressure relief valve and an inlet to the body and a retaining stand which supports the entire cell when under pressure. The cell has been previously modified by the addition of a 0.5 μm grade porous steel plate supplied by Mott Corp. (Farmington, Connecticut, USA) between the membrane and support plate to avoid any compaction effects of the membrane on top of the support plate channels by the exertion of pressure. The Sterlitech and Membranology cells operate at much higher pressures than the Amicon cell and therefore are constructed from stainless steel. The Sterltiech and Membranology cells both consist of a cylindrical body, a porous membrane support, a suspended magnetic stirrer, a solid steel base, a solid steel lid through which the nitrogen gas (driving force) is supplied and a pressure relief valve. The base and lid in both cases are held together using high pressure bolted clamps. In order to maintain isothermal operating conditions throughout the experimental work the Amicon cell was fitted with an external water jacket, while the Sterlitech and Membranology cells were immersed in a custom made water bath during operation. The three cells were set to the desired stirring rate and left to reach thermal equilibrium prior to starting experimental work.

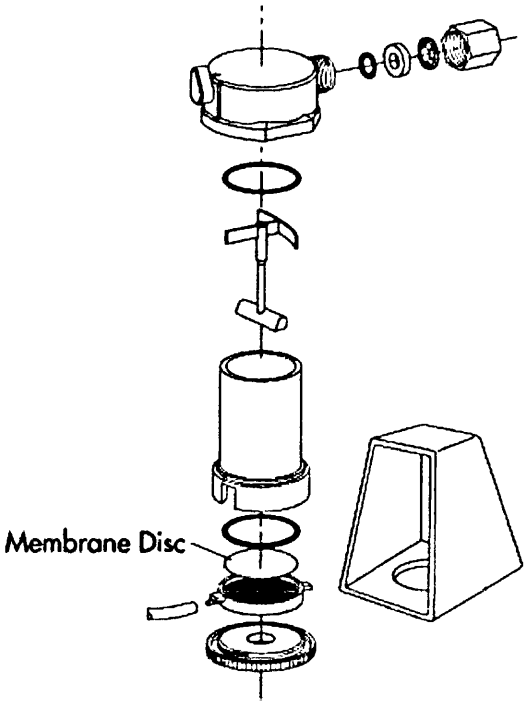

Figure 3.1: A schematic diagram of the Amicon™ 8050 stirred ultrafiltration cell

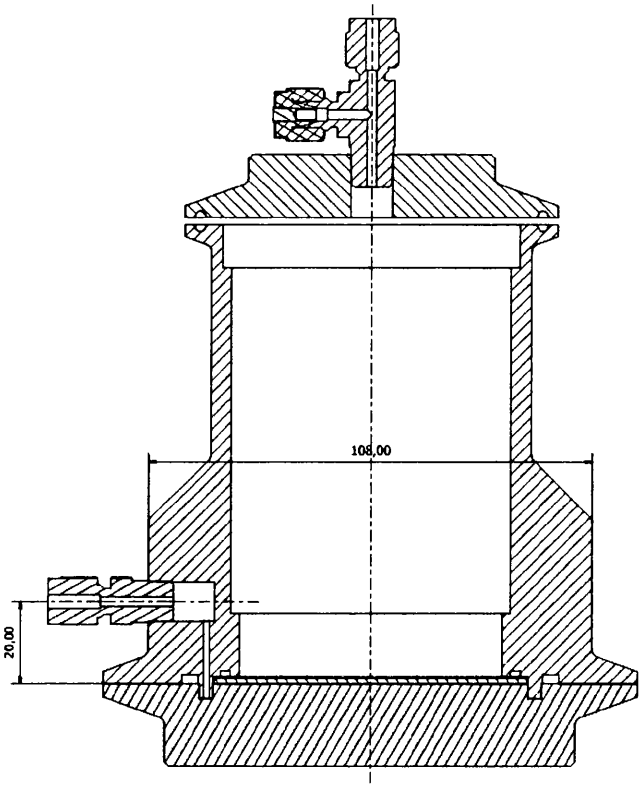

Figure 3.2: A schematic diagram of the Membranology stirred nanofiltration cell

The general setup of the three membrane cells used was similar and is shown in Figure 3.3.

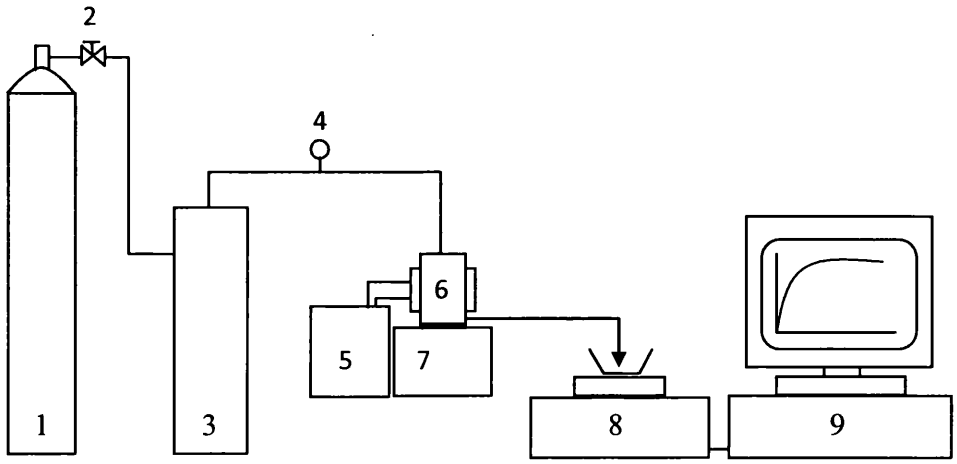

Figure 3.3: A schematic diagram of the dead-end filtration equipment. (1) nitrogen cylinder, (2) valve, (3) 1.5 L reservoir, (4) pressure sensor, (5) water bath, (6) Amicon cell, (7) magnetic stirrer, (8) electronic balance, (9) PC

For the operation of the Amicon cell, the membrane was placed on the porous steel plate and then slotted into the cell support plate. The O-ring gasket was carefully placed on the membrane peripheral surface and the cell support plate was inserted into the body of the cell and the quick fit base screwed on tight, completing the base assembly of the cell. The magnetic stirrer was then placed inside the body of the cell and the experimental solution poured in taking care to fill the cell to minimise the chance of vortexing. Next, the top cap was secured to the body and the cell was placed inside the retaining stand and set onto a magnetic stirring table and the stirrer speed set. The water jacket is then connected to the water bath and the temperature control unit switched on. The pressure relief valve was then set to the vertical closed position and the cell was pressurised using compressed oxygen-free nitrogen gas (BOC, UK) from a free standing cylinder. The applied pressure to the cell was measured using a digital pressure meter supplied by PSI-Tronix (Tulare, CF, U.S.A.).

For the operation of the Sterlitech and Membranology cell the set up procedure is the same. In both cases the cell was inverted before carefully placing the

membrane on the O-ring gasket prior to placing the support plate on top. The base plate is then placed on the base of the cell before securing tightly with the high pressure bolted clamps and the cell turned upright. The magnetic stirrer is lowered into the cell and the experimental solution added. The lid is then secured in place before placing the cell in the water bath above the magnetic stirrer. The stirrer speed is then set and the water bath switched on. The cell is then pressurised using compressed oxygen-free nitrogen gas (BOC, UK) from a free standing cylinder and the applied pressure monitored using a pressure sensor (Druck DPI 104, RS Components, UK).

For all three cells the permeate flux was measured by collection of the permeate over time and was recorded by mass via an electronic balance (Ohaus Navigator N24120, Ohaus Europe, Switzerland) connected to a personal computer running WinWedge capture software (TAL Technologies, Pennsylvania). The cells were maintained at 25 ± 0.5 °C by the attached water baths. On completion of the experiments, the nitrogen gas supply was cut and the pressure slowly released from the system by opening the pressure relief valve. The lids were then removed and the contents of the cell emptied. At this point, either the cell was thoroughly rinsed and fresh solution was poured into the cell and a new experiment started or the cell was dismantled and cleaned by reversing the set-up instructions.

All membranes were cut to size for the three cells and immersed overnight in ultra-pure water in darkness at 3 °C unless otherwise stated. Prior to starting any experimental runs each new membrane was operated at the maximum operating pressure of the experiments (unless otherwise stated) for 1 hour with ultra pure water obtained from an Elix3® Essential water purification system (Merck Millipore UK Ltd., Watford, UK).

The dead-volume beneath the membrane support plate was calculated to be approximately 6mL for the Sterlitech and Membranology cells and 2mL for the Amicon cell. Thus, the Sterlitech and Membranology cells were flushed with 10 mL, and the Amicon cell with 2 mL of feed before any samples were taken to ensure the dead-volume was cleared and each sample was representative. Furthermore, the

quantity of sample taken was kept to a minimum in order to reduce the concentration effect, i.e. so no more than 10% of the tank contents was removed in any given experimental run to ensure the feed concentration did not vary excessively.

3.2 Membrane Experimental Methods

The following section outlines the specific materials and methods used for the membrane experimentation work.

All laboratory scale rejection experiments were carried out using a frontal filtration set-up illustrated in Figure 3.3. For all laboratory based studies ultra pure reverse osmosis water was obtained from an Elix3® Essential water purification system (Merck Millipore UK Ltd., Watford, UK). For the pilot scale studies mains tap water was used in all cases.

3.2.1 Mass Transfer Study

The objective of this study was to investigate the concentration polarisation and mass transfer effects occurring in three commercially available frontal filtration membrane cells through the analysis of rejection data for poly ethylene glycol (PEG), an uncharged solute.

The three membrane cells used were the Amicon, Sterlitech and Membranology cells. The Nadir UH004 membrane (Microdyn Nadir, Germany) was used for the investigation which has a reported molecular weight cut off (MWCO) of 4000 Da. The UH004 membrane was selected as the pore size of the membrane would allow a wide range of PEGs to be used for the rejection study, in order to obtain a comprehensive data set for the mass transfer study. Characterisation using a single sized solute has been shown to be more accurate than the filtration of mixtures (Causserand et al. 2004) and so the solute used for this study was PEG 3400 (Sigma Aldrich, Dorset, UK) with a feed concentration of 600 mg/L. All experiments were conducted in frontal filtration mode, as illustrated in Figure 3.3. A concentration of 600 mg/L was selected to minimise solute-solute interactions and hence concentration polarisation effects occurring within the system. The concentration of 600 mg/l is also representative of that used in previous studies by Causserand et al. (2004) and Rohani et al. (2011), who used 1000 and 400 mg/L respectively.

The Amicon cell was operated at 1, 3 and 5 bar with a stirrer speed of 0, 60, 120, 180, 240 and 300 rpm; note that the maximum operating pressure for the Amicon cell is 5.17 bar. The Sterlitech and Membranology cells were operated at 5, 10, 20 and 30 bar with stirrer speeds of 0, 60, 120, 180, 240 and 300 rpm.

Prior to starting experimentation, membranes were pressurised for 1 hr at 5 bar with ultra-pure water for all three cells. Each experiment was conducted at least three times and the average result reported.

3.2.2 NTR-7450 Characterisation

The NTR-7450 membrane characterisation experiments were undertaken in the Membranology cell. The NTR 7450 membrane (Nitto Group, California, USA) was used for the investigation. This membrane was characterised due to the wide range of membrane properties reported in the literature, furthermore this membrane was used in both the fungal and potato bioseparation studies reported later (Chapter 5 and 6).

Two sugars, sucrose and raffinose and five PEGs (Polyethylene glycol, molecular weight: 200, 400, 600, 1000, 1450) were obtained in high purity form from Sigma Aldrich (Dorset, UK) and a feed concentration of 1 g/L for each solution was maintained throughout the experimental work. NaCl and $CaCl_2$ were obtained in high purity form (Fisher Scientific, Loughborough, UK) and the salt solutions were made up with ultrapure water to the required concentration. The NaCl salt concentrations used for the experimental work were 0.001, 0.01, 0.025 and0.1 M while the $CaCl_2$ concentration studied were 0.001 and 0.01 M. Salt rejections were studied at pH 3, 6 and 9, with the feed solution pH adjusted by the addition of 0.1 M HCl or 0.1 M NaOH, both obtained from Fisher Scientific (Fisher Scientific, Loughborough, UK).

The PEG rejection experiments were undertaken at 300 rpm a value optimised to reduce mass transfer and hence concentration polarisation effects (reported in chapter 3 - Mass Transfer Study). PEG rejection experiments were undertaken at 3, 5, 10, 20 and 30 bar with the operating temperature maintained at 25 °C. The salt

rejection experiments were undertaken at a feed pressure of 10 bar at three feed pH values of 3, 6 and 9, adjusted by the addition of 0.1M HCl and NaOH. In all cases a starting feed volume of 250 mL was used. Prior to starting experimentation, membranes were pressurised for 2 hours at 30 bar with ultra-pure water, with the efficiency of the membrane compaction also being investigated.

3.2.3 Hydrodynamic Drag

In this experiment the system consisted of only the Membranology cell (Membranology Ltd., Swansea, UK). The Nadir UH004 membrane was used for the investigation which has a reported MWCO (molecular weight cut off) of 4000 Da (Microdyn Nadir, Germany). The UH004 membrane was selected for this study as the pore size of the membrane would allow a wide range of PEGs to be studied in the rejection study. The purpose of this study is to examine the relationship between pore size and solute size and their subsequent rejections, therefore the membrane selected needed a range of PEGs both larger and smaller than the membrane pore size.

Sucrose and eight PEGs (Polyethylene glycol, molecular weight: 200, 400, 600, 1000, 1450, 2000, 3400, 4600) were obtained in high purity form from Sigma Aldrich (Dorset, UK) and a feed concentration of 600 mg/L for each PEG solution was maintained throughout the experimental work. Similarly for the sucrose solution. The cell was operated at 1, 3, 5, 10, 15, 20, 25 and 30 bar applied pressure with a stirrer speed set to 300 rpm (previously determined as optimum to account for mass transfer effects).

Prior to starting experimentation, the membrane was pressurised for 1 hr at 30 bar with ultra-pure water. Each experiment was conducted at least three times and the average result is reported.

3.2.4 Further understanding of Di-electric Exclusion

In this experiment only the Membranology (Membranology Ltd., UK) cell was used. The membrane used in this study was the Desal DK (GE Osmonics, USA). This membrane is a thin film composite with a polyamide active layer. The Desal DK membrane was selected for the dielectric study as the membrane has a high surface charge and a distinct isoelectric point at approximately pH 4. These properties make the membrane ideal for the study of dielectric propoerties as the pH can be easily adjusted to study salt rejection at the membrane isoelectric point.

All membrane experiments were carried out using a stirrer speed of 300 rpm. NaCl was obtained in high purity form (Fisher Scientific, Loughborough, UK) and salt solutions were made up with deionised water to the required concentration. The salt concentrations used for the experimental work were 0.000017, 0.0001, 0.00017, 0.0017, 0.00025, 0.001, 0.017, 0.01, 0.1, 0.6 M and were selected to cover a wide range typical of academic studies and relevant for industrial purposes. NaCl has been selected as this represents the bulk species within seawater and this range of concentration is the equivalent of 1 to 35,000 ppm with the highest concentration typical of seawater. Rejection was calculated from conductivity measurements of the feed and samples of the permeate.

3.2.5 Feasibility of membrane technologies as a technique for rapid isolation and purification of fungal bioactives

All membrane rejection experiments were carried out using the Membranology cell. All experiments were undertaken at 300 rpm.

Ultrafiltration (Prefiltration) of the culture filtrate and the operation was conducted at a constant pressure of 10.0 bar(g). A filtration volume of 250 mL was used throughout the ultrafiltration study. The membrane used was a 4000 MWCO polyethersulfone ultrafiltration membrane (Nadir UH004, Microdyn-Nadir, Wiesbaden, Germany). The UH004 membrane was selected for the pre-filtration of the culture filtrate as the membrane would reject compounds with a MW greater than 4000, thus resulting in a fraction with compounds below 4000 MW. The

compounds of interest have molecular weights below 1000 and as such should easily permeate the membrane.

Nanofiltration (NF) was conducted at a constant pressure of 20.0 bar(g) with a feed volume of 100 mL for each run, with the run terminated following the permeation of 50 mL. The membranes used for the experimental work were the Nitto-Denko NTR-7450 nanofiltration membrane (Nitto Denko UK Ltd., Mansfield, UK) and the NF 200 (Dow Filmtec) with MWCO of 1000 (Bargeman et al., 2005) and 300 (Bellona and Drewes, 2005) respectively. Both the nanofiltration retentate and permeate were then frozen at -40°C prior to analysis and further testing. The NTR-7450 and the NF200 membranes were selected for this experimental work such that one membrane would permeate and one membrane would reject the compounds of interest based on steric effects. The MWCO of the NTR-7450 membrane is 1000 and as such the bioactive compounds should be permeated while the MWCO of the NF200 is reported as 300 and therefore should retain all of the bioactive compounds above MW 200. Further information of membrane selection is presented in Chapter 5.

3.2.6 Generating a Value Stream from Potato Peel Waste

Laboratory Scale Equipment and Methods

The laboratory scale MF experiments were carried out in an Amicon 8050 frontal filtration cell (Millipore UK Ltd., Watford, UK). The operating volume was 50 mL and the stirrer was set to 300 rpm. Four applied pressures were studied, namely 0.1, 0.3, 0.5 and 1.0 bar. Five flat sheet microfiltration (MF) membranes were tested in the present study. The membranes used were all cellulose acetate membranes with 5, 1.2, 0.8, 0.2, and 0.05 μm pores (Millipore UK Ltd., Watford, UK). The MF membranes are selected in order to evaluate the ideal MF membrane size for the removal of particulates from the potato extract.

The laboratory scale nanofiltration (NF) experiments were undertaken in the Membranology cell as described in Chapter 3. A sample feed volume of 250 mL was

used throughout the experimentation. All membrane experiments were carried out using a stirrer speed of 300 rpm optimised through previous experimentation to reduce mass transfer effects. Five applied pressures were studied, namely 5, 10, 15, 20 and 30 bar.

Four commercially available nanofiltration membranes were studied, namely the NF 270 (Dow Filmtec, USA), NTR-7450 (Hydranautics, California, USA), Desal HL and the UF GE (Both GE Water & Process Technologies, Pennsylvania, USA) with reported molecular weight cut-offs of 300, 600-800, 150-300 and 1000 respectively. Prior to sampling 5 mL of sample was permeated to waste in order to allow for cell hold-up, followed by the collection of a 20 mL sample. This range of NF membranes was selected to cover the size range required, i.e. 150 - 1000 Da, with the aim of developing the optimum separation with high separation efficiency while simultaneously achieving the highest flux.

Pilot Scale Equipment and Methods

The pilot scale equipment employed in this study was custom built, with both the MF and NF equipment designed so that a single industrial membrane module could be used without modification. The configuration of the apparatus is presented schematically in Figure 3.4 and 3.6. Photographs of the apparatus are shown in Figures 3.5 and 3.7.

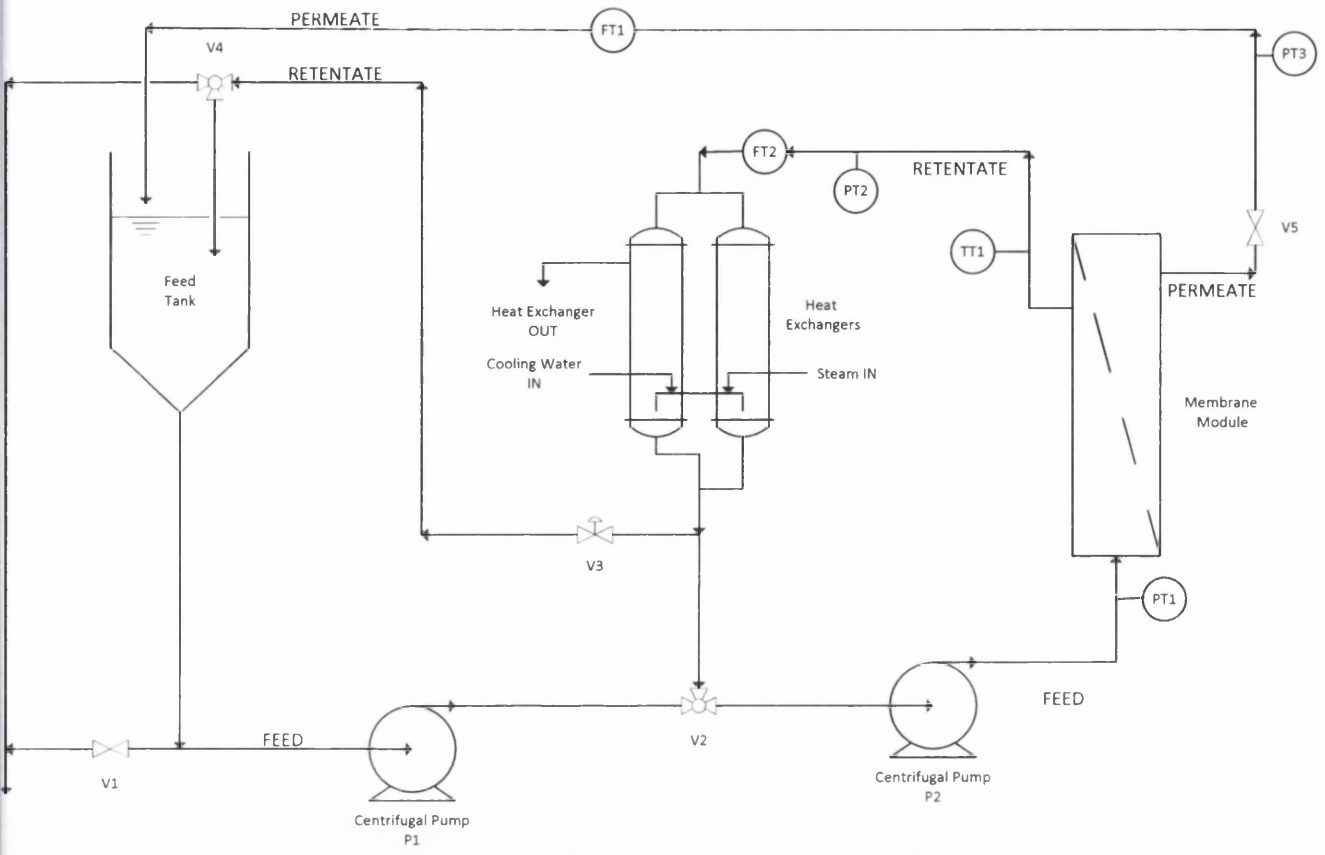

Figure 3.4: P&ID of Pilot Scale Microfiltration System

Figure 3.5: Photo of the pilot scale microfiltration system used in the experiments.

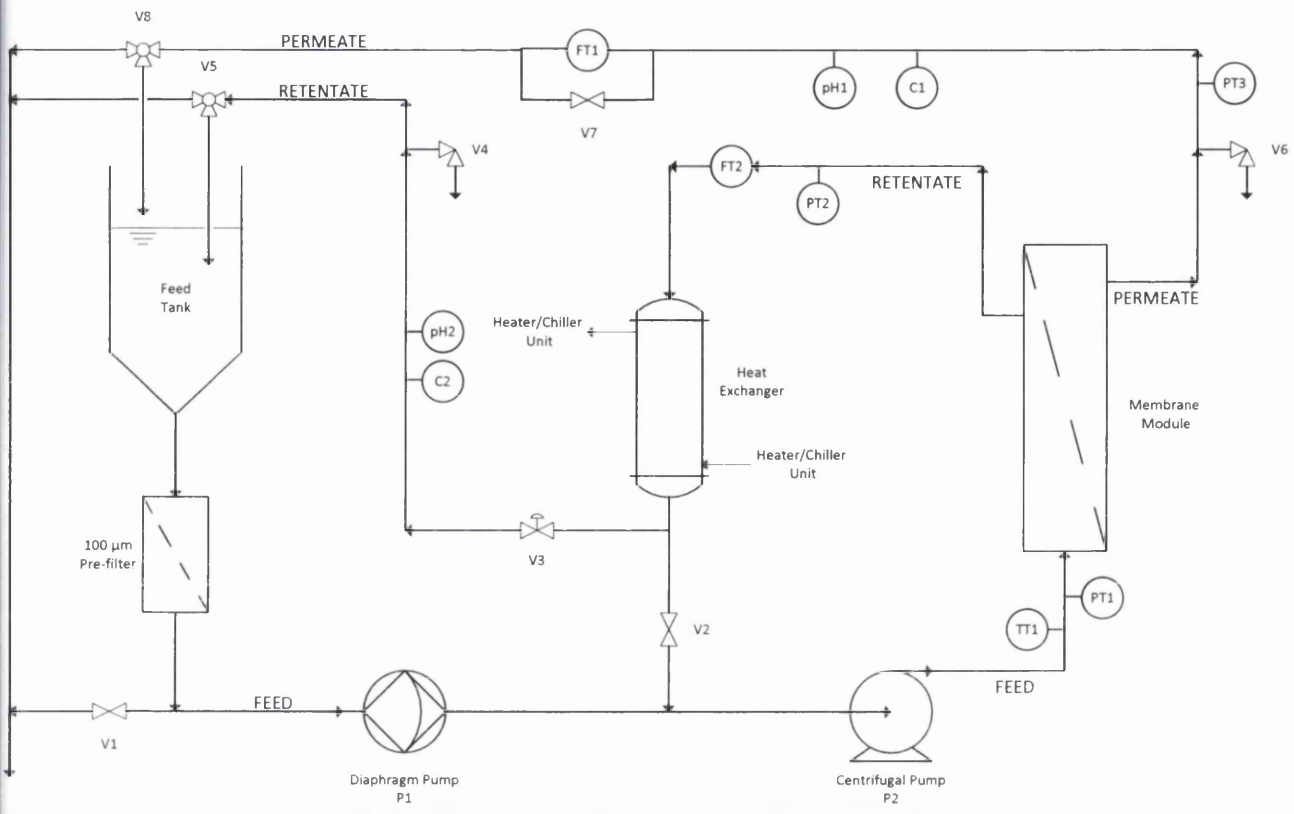

Figure 3.6: P&ID of Pilot Scale Nanofiltration System

Figure 3.7: Photo of the pilot scale nanofiltration system used in the experiments.

Microfiltration

The microfiltration equipment shown in Figure 3.4 under normal process conditions operates with valve V2 open, running the system as a feed and bleed setup, with a high cross-flow velocity being generated by pump P2. The system used is over-engineered for the application required, however the use of two pumps allows the pressure and cross-flow velocity to be independently controlled which is beneficial in a research environment. The system was originally designed to be operated as both a micro- and ultra-filtration system, capable of operating at pressures of up to 6 bar, however, the system is capable of operating at the much lower pressures required for microfiltration through the use of inverters for the pumps. The system consists of a 100 litre stainless steel feed tank which feeds into the feed pump (P1: Fristam FPE 742, Fristam Pumpen KG, Germany) which is capable of delivering up to 5.5 bar pressure at a flowrate of up to 10 m^3hr. The flow then enters the second pump (P2: Fristam FPE 722, Fristam Pumpen KG, Germany) which is capable of delivering up to 3.8 bar at a flow rate of up to 10 m^3/hr. The flow from P2 then passes through the membrane module. The retentate flows out of the membrane module and enters the heat exchanger system. The heat exchangers consists of two shell and tube heat exchangers connected to a cold water and steam supply. The steam supply is cracked open and the cold water supply is controlled by the use of a solenoid (Burkert 6213 A, Burkert, Germany) connected to a thermostat on the control board. The retentate re-circulates around the loop through V2, with the retentate returning to the feed tank controlled by V3.

An industrial scale 0.2 µm polymeric microfiltration hollow fiber membrane was used throughout the pilot scale microfiltration study (Model number: CFP-2-G-55, GE Healthcare & Life Sciences, Buckinghamshire, UK). The membrane is made from polysulfone and has an active area of 1.8 m^2 and is capable of handling temperatures of up to 80°C and a pH range of 2-13. The system was operated at a number of pressures controlled via the pumps (P1 and P2) and valve V3. The pressures used in the study were 0.5, 1.0 and 1.5 bar. A temperature of 20°C was maintained throughout the experimental work controlled by the solenoid valves attached to the heat exchangers controlling the steam and water flow rates.

Nanofiltration

The nanofiltration equipment shown in Figure 3.6 under normal process conditions operates with valve V2 open, essentially running the system as a recirculation loop, with the high cross-flows being generated using P2. In order to produce a high enough operating pressure for nanofiltration separations pump P1 operates as an injector pump, adding new high pressure feed from the tank into the recirculation loop. Since the flow into the loop is greater than that of the permeate flow rate, some material must flow through the needle valve (V3) back into the feed tank in order to satisfy a material balance and prevent accumulation within the recirculation loop.

The scale of the majority of cross-flow research experiments means that a single pump is often used. However, from a research perspective, the use of two pumps is advantageous in that it facilitates independent control of applied pressure and cross-flow velocity. For a single pump, pressure and flow are inter-related which means that independent control of inlet feed pressure is not possible. The inclusion of two pumps allows the variation in cross-flow at a constant pressure, therefore allowing the mass transfer (and hence concentration polarisation) characteristics of the system to be investigated. In pilot experimental work the rejection of material with varying pressures is often studied therefore maintaining a constant cross-flow velocity is vital. Furthermore, independent variation of cross-flow velocity at a constant pressure allows a greater degree of control to prevent membrane fouling i.e. high cross-flow rate to scour membrane surface and remove deposited materials, both under operation and cleaning regimes.

P1 is a Wanner Hydra-Cell diaphragm pump capable of raising the feed pressure up to 60 bar at a pressure dependent supply of up to 0.3 m^3h^{-1}. Feed pressure is controlled through speed control of the motor using the inverter. P2 is a Fristam centrifugal pump that can generate flows of up to 2.5 m^3h^{-1}. The pump is designed to withstand inlet pressures of up to 60 bar and is also speed-controlled via an inverter. The fine adjustments of the pressure are undertaken by adjustment of the needle valve, V3.

Volumetric flows were measured on both the retentate and permeate sides of the membrane using Endress and Hauser Promag 33F flowmeters, shown as FT2 and FT1 respectively. The flow meters operated on the principle of a potential being induced between a pair of electrodes as the conductive medium flows through the magnetic field. The induced potential was proportional to the flow velocity (which is converted to a volumetric flow). The detection limit of the flowmeters reported by the manufacturers is 5 $\mu S\ cm^{-1}$ therefore the pure water flux measurements were measured manually using a volumetric flask and a stopwatch.

Operating pressures were measured at three points in the system, namely the module inlet (feed pressure), retentate and permeate lines, therefore allowing the TMP to be calculated. In each case Wika Instruments stainless steel, diaphragm-type transmitters were used and the signals fed to panel mounted displays, with the precision of the instruments being ± 0. 1 bar.

Membrane flux rates are highly dependent on temperature, therefore temperature control is vital in research experiments. The system temperature was monitored using a Thermodata Components temperature sensor on the feed line, labelled TT1. The signal was sent to the display panel and regulated by connecting the heat exchanger to a heater chiller unit. The displayed reading was accurate to within ± 0.2°C.

The setup is also capable of measuring conductivity (C1, C2) and pH (pH1, pH2) of the system, however the results were not used in the analysis and therefore will not be described.

An industrial scale NTR-7450 spiral-wound membrane was used throughout the pilot scale nanofiltration study (Hydranautics, Oceanside, California, USA). The membrane is made from sulfonated polyethersulphone and has an active area of 1.4 m^2 and is capable of handling temperatures of up to 60°C, a pH range of 2-11 and a maximum pressure of 40 bar. The system was operated at a number of pressures controlled via the pumps (P1 and P2) and valve V3. The pressures used in the study were 10, 20 bar. A temperature of 20°C was maintained throughout the

experimental work controlled by the solenoid valves attached to the heat exchangers controlling the steam and water flow rates.

Pilot scale membrane cleaning methodology

Industrial scale membrane modules are expensive and it is essential that these membranes are re-used or the life-span maximised where possible. Therefore, an appropriate and effective cleaning protocol is essential in order to maintain membrane performance over time. In this study, the first stage in the cleaning process of both the pilot MF and NF plants was a rinse with clean water (approximately 30 litres). This rinse stage is designed to remove any bulk foulants that may be present on the membrane surface. This cleaning step was undertaken at a low pressure in order to prevent any unnecessary abrasion of the membrane fouling. The initial rinse was repeated until the water in the recycle tank was clear (approximately 2-3 times). The second cleaning stage for both the MF and NF membranes was to fill each of the rigs with 30 litres of clean ultra-pure water (RO water). The water (25°C) was re-circulated and NaOH slowly added until the pH reached 10.5. The alkaline cleaning stage is known to remove organic fouling. The NaOH was re-circulated for a period of 30 minutes at a low pressure (i.e. no permeate produced to avoid driving foulants towards pores) before the system is drained and rinsed with volumes of fresh water until the pH was neutral. The third cleaning phase undertaken was the addition of citric acid to water (25°C) to produce a 2% solution. This solution is designed to remove any mild inorganic fouling present. The solution was re-circulated at a low pressure (i.e. no permeate produced) for a period of 30 minutes before draining and thoroughly rinsing the system until the pH is neutral. The membranes clean water flux (CWF) was then determined and compared to that prior to experimentation to ensure successful cleaning had taken place. Further more specific membrane cleaning information is included in Chapter 6.

3.3 Analysis Methods

3.3.1 UV-Vis and TOC Analysis

Sample analysis of PEG was carried out using a Varian Prostar 350 Refractive Index Detector (Agilent Technologies, Wokingham, UK). The PEG solutions were pumped through the detector using an Agilent 1100 (Agilent Technologies, UK) HPLC Pump and the signal generated was recorded. This signal value was then converted to the permeate PEG concentration by comparison to a calibration curve of known

concentration. Sucrose analysis was carried out using a Shimadzu TOC-L CPH analyser (Shimadzu UK Ltd. Milton Keynes, UK) according to the manufacturer's instructions.

For the characterisation of the NTR-7450 membrane both the PEG and sugar analysis was carried out using the Shimadzu TOC-L CPH analyser according to the manufacturer instructions.

3.3.2 Conductivity measurements

All conductivity measurements were performed at 25 °C using a Russell RL060C conductivity meter and probe (Fisher Scientific, Loughborough, UK). pH was measured using an IQ 150 pH meter and probe (Spectrum Technologies Inc., Illinois, USA). Salt rejection was calculated from conductivity measurements of both the feed and samples of the permeate. Conductivity is a measure of the ability of a liquid to carry an electric current, usually measured using a probe with two electrodes a known distance apart where an electrical potential is applied which measures the electrical resistance of the fluid. In order to obtain an accurate measurement it is essential that the probe is calibrated before use. The conductivity measurement is not necessarily linear over the entire range of concentration of interest, therefore the meter should only be used within the range calibrated. Furthermore the cell constant of the probe must be known as well as the conductivity factor of the ions in solution as current is carried to a different degree by cations and anions. The probes used in this study had cell constant values of 1 for high conductivity solutions and 0.1 for low conductivity solutions. Similarly a pH meter measures the concentration of hydrogen ions present (measuring range usually pH 1-14) and due to the tendency of the instrument to drift pH probes and meters must be calibrated prior to use through the generation of a two-point or three-point calibration curve using known standards.

3.3.3 Electrokinetic Study

The zeta potential (ζ-potential) was determined using an electrokinetic analyser (EKA, Anton Paar, Graz, Austria) based on the streaming potential method (Oatley et al., 2012). Flat sheets of the NTR-7450 and Desal DK membrane were studied over a range of pH and concentrations in order to generate a zeta profile for the membrane from which the isoelectric point is determined. The feed solution pH was adjusted via the addition of 0.1 M NaOH and HCl (Fisher Scientific, Loughborough, UK). The ζ-potential was calculated using the Smoluchowski equation:

$$\frac{\Delta E}{\Delta P} = \frac{\varepsilon_r \varepsilon_0}{\eta \lambda} \zeta$$

where ε_r is the dielectric constant of fluid, ε_0 is the permittivity of free space, η is the viscosity of the fluid and λ is the bulk conductivity.

In the case of the Desal DK membrane the membrane isoelectric point was additionally determined from salt rejection studies at varying pH values. The feed solution pH was adjusted by the addition of 0.1 M HCl or 0.1 M NaOH, both obtained from Fisher Scientific (Fisher Scientific, Loughborough, UK). The pH was varied between pH 3 to 10 using each of the concentrations stated previously. The isoelectric point was determined from the minimum rejection value obtained over the range of pH at fixed concentration and a constant applied pressure of 10 bar.

All zeta potential experiments were conducted on a minimum of ten measurements and the average result reported.

3.3.4 Particle Sizing (ZetaSizer)

Particle size for the PEG solutions was determined using a Zetasizer (Nano ZS, Malvern Instruments, Malvern, Worcs., UK.). Samples were prepared using deionised water and were filtered with a 0.22 µm syringe driven filter unit (Millipore U.K Ltd.) into a 4.0 mL glass cuvette (Malvern Instruments, Malvern, Worcs., UK) for ZetaSizer measurements. The method and number of

measurements performed was selected in accordance to British Standard 3406-8:1997. For a particle size of less than 10 nm, a concentration greater than 0.5 g/litre is recommended with no upper limit providing the material exhibits no aggregation or gelation. In this study a concentration of 4 g/litre was used in all cases except for molecular weights below 600 where a concentration of 10 g/litre was used in order to improve measurement intensity and count rate. The data obtained was compared to literature values where possible and theoretical models for validation.

Particle size measurements and distribution measurements for the fungal and potato samples were made using dynamic light scattering (DLS) measurements using the High Performance Particle Sizer with NIBS Technology from Malvern (Malvern Instruments, Malvern, UK).

3.3.5 Scanning Electron Microscopy (SEM) Imaging

Scanning electron microscopy imaging was performed using an Hitachi S4800 Scanning Electron Microscope (Hitachi High Technologies America, Inc., CA, USA). The membrane samples were dried and fractured by quenching in liquid nitrogen (freeze-fractured) and gilded with gold under vacuum prior to observation. The membranes were freeze fractured in accordance of the 'Direct Freeze Fracture' method by Ferlita et al. (2008). A gold layer of 5-10 nm thickness was deposited on the membrane surface using a sputter coater (Edwards S150B Sputter Coater) in order to increase membrane conductivity. In all cases the cleaved edge of the membrane was examined perpendicular to the cut plane. An acceleration voltage of 1 kV and the use of the lower detector was found to provide images with the lowest charge effects.

3.3.6 Brunauer-Emmett-Teller (BET) nitrogen adsorption

Membrane structural characteristics have been previously determined using gas adsoprtion-desorption (Calvo et al. 1995; Prádanos et al. 1996; Fang et al. 2014). Pore size measurements were determined using a Quantachrome Nova 2000e surface area analyser (Quantachrome UK Ltd., Hook, UK). The measurement

technique of this instrument is based on physisorption and desorption of a gas, nitrogen in this case, at the surface and inside the pores of the sample. Prior to analysis, the two membrane samples were prepared by degassing under vacuum at 50°C for 48 hours until a constant weight was obtained. The weight of the dry sample and sample tube were determined using an analytical balance. In order to determine the sample properties, the sample cell is placed under vacuum and immersed in liquid nitrogen; a known flux of nitrogen is then introduced to the cell while simultaneously measuring the pressure. Pore size data were calculated by applying the Horvath-Kawazoe (HK) method (Fang et al. 2014).

3.3.7 Atomic-Force Microscopy (AFM) measurements

Membrane pores size was also determined using AFM and measurements were performed on a Multimode AFM with Nanoscope IIIa controller (Bruker, USA) using manufacturer supplied software. All measurements were carried out using tapping mode under ambient laboratory conditions (humidity ~60%, temperature ~22°C) or in high purity water (resistivity 18.2 MΩ s) using TESP tapping mode levers for measurements in air (Bruker, nominal spring constant 20-80 N/m) and contact mode NP-S levers for measurements in liquid (Bruker, nominal spring constant 0.12 N/m). Data was analysed using the instrument control software (NanoScope software version 5.31R1). The particle size analysis feature of the AFM software was employed to measure the size of surface pores. This software facilitates the measurement of features sited higher than an arbitrary threshold set by the user. To measure the size of channels (interpreted as pores) negative thresholding was selected. As the value of the threshold used is arbitrary, a value of 0 was employed in all studies, i.e. the mean plane for the image was used as the baseline. The software then generated data for the mean diameter, mean area (along with standard deviations) and count number for the channels, i.e. for all features identified as being below the threshold. By multiplying the mean area by the count number the total surface area of the pores in the image was estimated and porosity was determined as a percentage of the total surface of the image occupied by pores.

3.3.8 Moisture content determination

Moisture content of the potatoes was measured by drying the samples using a freeze dryer in order to minimise any alteration in potato composition during the drying of samples (Bártová and Bárta, 2008). All samples were weighed then frozen at -40 °C for a minimum of 6 hours prior to freeze drying (Thermo-Scientific) for 48 hours then re-weighed to calculate moisture loss. Samples were then crushed to a fine powder with a pestle and mortar and the resultant powder stored in tightly sealed plastic vials at – 40 °C. In this study only peel content is analysed and not the whole un-peeled tuber.

3.3.9 Protein Analysis

The quantity of protein present in the potato samples was analysed using the Lowry assay (Lowry et al. 1951). The potato samples required extraction and solubilisation using NaOH (Snyder and Desborough, 1978; Pinto et al. 1993). The protein was extracted by adding 5 mL of 0.5M NaOH to 100 mg of freeze dried potato powder, this was then incubated for 2.5 hours at 30 °C. A 0.5 mL sample of this extract solution was then placed in a 10 mL boiling tube before adding 0.7 mL of Lowry solution (All chemicals obtained from Sigma-Aldrich, Dorset, UK). The tubes were capped, vortexed and then incubated for 20 minutes at room temperature (22 °C) in darkness. Fresh 2M Folin-Ciocalteu reagent (Sigma-Aldrich, Dorset, UK) was prepared and 0.1 mL was added to each tube and vigorously mixed (Vortex Genie 2, Scientific Industries, Inc, New York, USA). Samples were then further incubated at room temperature in darkness for 30 minutes before vigorous mixing to prevent any colour gradients forming and transferred to a 1.3 mL micro-cuvette. The samples were analysed at 750 nm using a spectrophotometer (Unicam UV 300, ThermoScientific, Loughborough, UK). A calibration graph was created using known concentrations of bovine serum albumin (BSA) (Sigma-Aldrich, Dorset, UK) in order to calculate the quantity of total protein present.

3.3.10 Sugar and Starch Analysis

The concentration of total sugar and starch was determined using the Dubois method (Dubois et al. 1956) also known as the phenol-sulphuric acid method. 100 mg of the freeze dried potato powder samples was weighed out in triplicate into a boiling tube. Then, 5 mL of 2.5 M Hydrochloric acid (Sigma Aldrich, Dorset, UK) was added to the tube. The samples were then hydrolysed by placing the boiling tubes in a boiling water (~100°C) bath for 3 hours. The samples were then neutralised by the slow addition of sodium carbonate until effervescence ceased (VWR International, Lutterworth, UK) and made up to 50 mL with ultra pure water before centrifuging (Sorvall Biofuge Stratos, ThermoScientific, Loughborough, UK) at 10,000 rpm for 10 minutes. 0.1 mL of the centrifuge supernatant was then made up to 1 mL with ultra pure water. 3 mL of 98% sulphuric acid (Sigma Aldrich, Dorset, UK) was added to each tube followed by 1 mL of 5% phenol (Sigma Aldrich, Dorset, UK) before thoroughly mixing. The samples were then placed in a 30°C water bath for 20 minutes before being vigorously mixed again to avoid the formation of a colour gradient. The absorbance was then read using a spectrophotometer (Unicam UV 300, ThermoScientific, Loughborough, UK) set at 490 nm. A standard graph was created using known concentrations of glucose (Fisher Scientific, Loughborough, UK) in order to calculate the quantity of total carbohydrate present.

3.3.11 TOC Analysis

Total organic carbon (TOC) analysis was carried out using a Shimadzu TOC-L CPH analyser (Shimadzu UK Ltd. Milton Keynes, UK) according to the manufacturer instructions.

3.3.12 Calystegine Analysis

Calystegine were analysed according to the method described in Chapter 6.1.2.

3.3.13 Experimental Error

All experiments were performed in triplicate unless specifically stated otherwise.

3.4 Mass transfer study of three commercial frontal filtration membrane systems and comparisons of experimentally derived mass transfer values to theoretical correlations

A mass transfer study of the three membrane cells used within this study was performed. Understanding the mass transfer characteristics of the membrane equipment being used is essential in the accurate determination of membrane rejection properties as the difference between observed and real rejection may be significant. However, once mass transfer is accounted for the real rejection in all equipment will be similar and provides a methodology for scale up between small flat sheet studies to industrial modules. Figure 3.8 displays the experimental mass transfer coefficients obtained for the three cells studied.

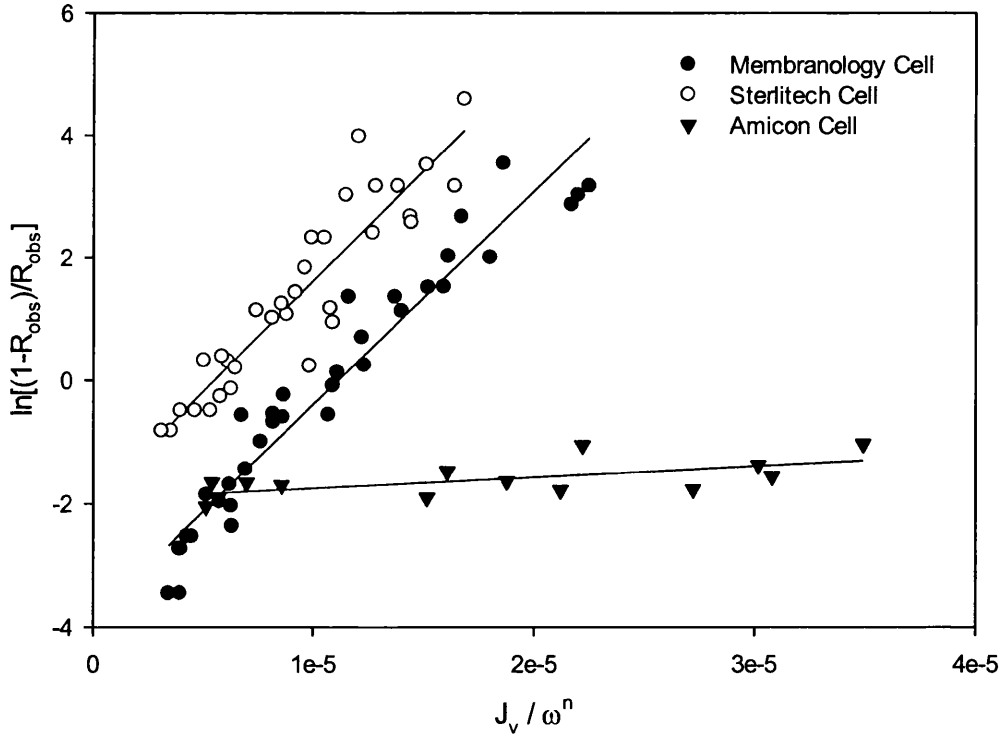

Figure 3.8: Linear analysis to determine the mass transfer coefficients of the Membranology, Sterlitech and Amicon membrane cells used in this study.

The data obtained from the mass transfer characterisation of the three membrane cells showed minimal mass transfer effects for the Amicon cell, while the Sterlitech and Membranology cells displayed much greater mass transfer effects. The results showed that a stirrer speed of 300 rpm is optimal in order to minimise the mass transfer effects in the systems studied. For further information on the mass transfer effects of the three membrane cells see Appendix A1.

3.5 Characterisation of the NTR-7450 Nanofiltration Membrane

Characterisation of the physical properties of a membrane is essential to achieve successful implementation of a membrane separation process. In the case of nanofiltration, the main physical properties of importance are the molecular weight cut-off (MWCO) or nominal salt rejection value for a mono-valent (mostly NaCl) or di-valent salt. MWCO is a correlation of observed rejection against a series of uncharged solutes with increasing molecular weight. The molecular weight cut-off is normally reported as the molecular weight of a solute, at which 90% rejection occurs (Van der Bruggen and Vandecasteele, 2002). The NTR-7450 membrane was used throughout this study and was characterised in order to better understand the membrane phisical properties. The NTR-7450 has been well characterised in the literature, however, there is significant variation in the MWCO reported. Figure 3.9 displays the membrane MWCO obtained from the filtration of PEGs.

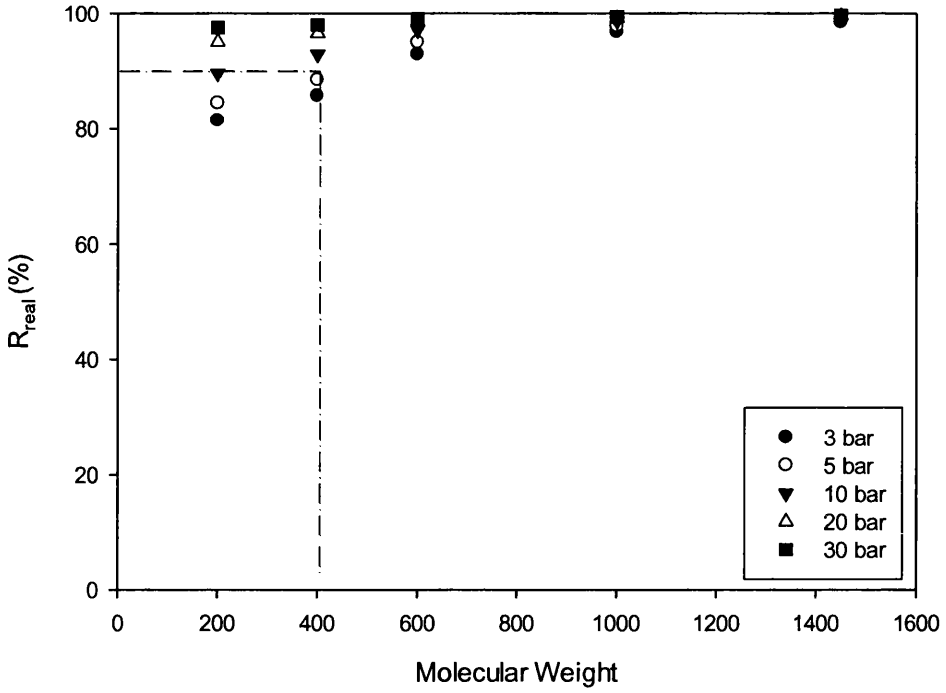

Figure 3.9: Real rejection versus PEG molecular weight

The MWCO obtained through this experimental work for the NTR-7450 membrane is 400 Da. For further information regarding the characterisation of the NTR-7450 membrane see Appendix A2.

4.0 Contribution to Nanofiltration Modelling

For traditional separation processes there are widely available process design methodologies for the design, scale up and optimisation and despite many developments in the understanding of nanofiltration in the last 30 years the understanding of the technology has not yet been exhausted. Accurate predictive models are essential for the widespread application of nanofiltration membranes, helping reduce developmental risk and time, allowing the technology to be evaluated prior to starting any experimental trials.

In this chapter two areas of nanofiltration membrane theory have been studied. The first area of study concerns the hindrance factors and comparison to theoretical correlations for nanofiltration. The second area concerns the current best practice for modelling nanofiltration and reverse osmosis using the extended Nernst-Planck equation and the role of dielectric exclusion.

Since the dawn of membrane technology there has been attempts made to understand and describe the transport through membranes. The apparent operating techniques of microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) would seem similar but the latter three (UF, and in particular NF & RO) do not separate by size alone (Cheryan. 1998). Microfiltration and to a certain degree ultrafiltration are separations that take advantage of the differing physical sizes of individual molecules to separate from a solvent through the application of a hydrostatic pressure (Bowen & Jenner, 1995). These separations techniques are often referred to as sieve filtration due to the overwhelming effect of size on the separation.

The prediction of nanofiltration transport mechanisms are seen as vital for process feasibility and optimization (Lefebvre et al. 2004). Model development is vital for membrane performance prediction, through shortening process selection by lessening laborious screening. The field of application of nanofiltration technology could be widened if quantitative methods for predicting process performance were available and accessible (Bowen & Mukhtar, 1996).

The development of nanofiltration membranes in the late 1980's required further development of the ultrafiltration models to account for the phenomena observed with nanofiltration separations. The size range at which nanofiltration operates, in between ultrafiltration and reverse osmosis, requires understanding of the interactions at the nanoscale. The last 20 years have seen the a large number of predictive models based on either empirical black box models based on irreversible thermodynamics (Levenstein et al. 1996), models based on the extended Nernst-Planck equation (Tsuru et al. 1991) a model which allows for membrane properties, a model based on charged capillaries (Jacazio et al. 1972) or a model based on the solution diffusion mechanism (Merten, 1963; Lonsdale et al, 1965; Paul, 1976). Despite recent technological advances, the implementation of nanofiltration at a large industrial scale faces ongoing difficulties (flux decline due to fouling), although the recent years have seen much greater uptake of the technology (Mohammad et al., 2015).

The separation mechanisms of nanofiltration involve steric (sieving) and electrical (Donnan) effects, a combination which allows nanofiltration to be effectively used for the separations of mixtures of charged and uncharged organic molecules and salts (Bowen and Mohammad, 1998; Bandini and Vezzani, 2003). Fundamentally, nanofiltration is a very complex process, with the events leading to rejection at the nanofiltration membrane surface taking place on a length scale in the order of one nanometre. At this near atomic scale the description of NF separation of charged and uncharged solutes using macroscopic descriptions of ion transport and partitioning break down due to the nano-scale phenomena involved. The ultimate goal is to produce and develop models that convey a fundamental understanding and simple quantification of the governing phenomena in such a manner that the theory may be utilised for industrial application (Bowen & Welfoot, 2005). A comprehensive predictive model will allow end users to predict process performance and membrane behaviour through use of membrane and system characteristics, resulting in smaller number of experiments, allowing for simpler process design and optimisation (Hilal et al., 2003).

The Donnan-Steric Pore Model (DSPM) proposed by Bowen et al. (1997) has been shown to successfully predict the NF performance in a dye-salt diafiltration (Bowen and Mohammad, 1998). The model uses the extended Nernst-Planck equation, modified to include hindered transport, with equilibrium partitioning to adequately describe the combination of electrical (Donnan) and sieving (steric) mechanisms observed. Bowen and Welfoot (2002) presented an updated version of the DPSM model which includes a dielectric exclusion term in the equilibrium partitioning expression. Due to the simplicity of the model, it is widely recognised that the coupling between equilibrium partitioning at the membrane/solution interface and the transport within the pores described by the extended Nernst-Planck equation is the most convenient for multi-component systems (Déon et al. 2013).

A major limitation in all NF models is the requirement of characteristic model parameters which are not readily measured at the near atomic length scale. The lack of detailed knowledge of the physical structure and electrical properties of real nanofiltration membranes is a stumbling point of NF modelling. The physical measurement of key characteristics is paramount to enable thorough understanding of NF separation processes, and therefore rely on improvements and developments in measurement techniques employed for the characterisation of NF membranes. The physical measurement of the physical characteristics would allow validation of currently assumed model parameters.

Following on from the argument on which model best describes NF membrane performance is whether real NF membranes have distinct pores. The discovery of distinct pores within NF membranes would clarify the confusion in the early NF models because the assumption of either a porous of homogeneous membrane structure could not be easily validated (Bowen and Mukhtar; 1996). A significant discovery in this respect has been atomic force microscopy (AFM), increasingly used to provide ultra-high resolution images of industrial membranes (Binning et al., 1986).

Wijmans & Baker (1995) suggest as a rule of thumb, the transition between permanent (pore flow) and transient (solution-diffusion) flow is in the range of 5 to

10 Å diameter. The presence of pores in the region of 1 nm in NF membranes is widely accepted (Bowen et al., 1997; Schaep et al., 1998; Bowen and Welfoot, 2002; Otero et al.. 2008; Senthil Kumar et al.. 2013), and therefore a pore flow model will be used to describe the ion transport within this thesis, namely the Updated Donnan-Steric Partioning Model (UDSPM) (Bowen & Welfoot, 2002), which is based upon the original Donnan-Steric Partioning model by Bowen et al. (1997).

Initial descriptions of particle transport through a NF membrane were based on irreversible thermodynamics, with the model first described by Kedem and Katchalsky (1963) and Spiegler and Kedem (1966) when studying transport through a RO membrane. The main issue surrounding the models based on the irreversible thermodynamics is that membranes were considered as a black box, and therefore do not consider the structural and charge properties of the membrane, and are consequently invalid for the prediction of nanofiltration performance. However, Perry and Linder (1989) (in their description of negative ion rejection) and Schirg and Widmer (1992) (in their study of dye-salt separation) proved that successful prediction of separation performance is possible using this approach (Bowen and Welfoot, 2002).

Tsuru and co-workers (Tsuru et al., 1991; 1991a) were among the first to develop a substantive model of electrolyte transport in charged porous UF and RO membranes based on the extended Nernst-Planck equations. The possible importance of volume flux in ion transport and modified the fixed-charge Teorell-Meyer-Sievers (TMS) model (Teorell, 1951, Meyer and Sievers 1936) used in equilibrium studies of ion-exchange membranes was highlighted by Tsuru et al., (1991; 1991a). Equilibrium partitioning at the pore inlet and outlet was included through a Donnan expression and the model was successful in describing the rejection characteristics of binary and ternary electrolyte mixtures. Their model contained two adjustable parameters, the effective membrane charge density, X_d, and membrane thickness, Δx. The governing equation for the flux of ions was given as:

$$j_i = -D_{i,p}\frac{dc_i}{dx} - \frac{z_i c_i D_{i,p}}{RT} F \frac{d\psi}{dx} + c_i V \qquad (4.1)$$

Bowen and Mukhtar (1996) later used a similar model but incorporated hindrance factors to account for the hindered nature of the movement of ions inside the membrane. The model was solved as if the membrane were homogenous (non-porous) but hindrance factors for diffusion and convection were included to allow for the transport of ions in the membrane taking place within a confined space. They found that the inclusion of the hindrance factors improved the accuracy of the model when fitted to experimental data while also allowing the determination of the effective pore radius of the membrane (in addition to the effective charge density and effective membrane thickness).

The nano-scale phenomena involved in uncharged solute and salt separations by NF are extremely complex and, as such, likely to be a rigorous test of any macroscopic description of ion transport and partitioning. The transport of uncharged solutes is reasonably well established through numerous studies of UF membranes. There have been many works over the past thirty years on modelling the transport of charged solutes across a charged membrane.

The space charge model (SCM) originally proposed by Gross and Osterle (1968) is a more rigorous (and complex) model that takes into account a radial distribution of both concentration and electric potential. Ion transport within the pores is described by the extended Nernst-Planck equation and pore volume flow by the Navier-Stokes equations. Ions are treated as point charges (i.e. no steric effects due to the ion sizes) and total electric potential, $\overline{\Psi}$, is subdivided into two parts as follows:

$$\overline{\Psi} = \Psi(x,r) + \psi(x) \qquad (4.2)$$

Ψ originates from the surface charge of the capillaries and ψ is due to the streaming potential in the *x* direction. For a small axial variation in potential (Wang *et al.*, 1995a), the radial distribution of concentration, $c_i(x,r)$, can be

calculated from the Poisson-Boltzmann equation using the electric potential, $\Psi(x,r)$, and a reference concentration, $c_i(x)$:

$$c_i(x,r) = c_i(x)\exp\left(-\frac{z_i F}{RT}\Psi(x,r)\right) \tag{4.3}$$

The extended Nernst-Planck equation, Eq. (4.1), then becomes:

$$j_i = \exp\left(-\frac{z_i F}{RT}\Psi\right)\left[Vc_i - D_i\left(\frac{dc_i}{dx} + \frac{z_i c_i F}{RT}\frac{d\psi}{dx}\right)\right] \tag{4.4}$$

The solution of this system of equations to obtain ion fluxes requires the use of complex numerical techniques and model parameters that are not measurable although the solution can be simplified by the assumption of a Hagen-Poiseuille velocity distribution inside the pore (Jacazio *et al.,* (1972). Recent workers have attempted to develop this space-charge approach. (Hall *et al.*, 1997) formulated a rigorous model of multi-component ion transport in RO membranes where specific ion interactions were included in partitioning and H^+ and OH^- transport was included in a chemical model for membrane charge formation. Basu and Sharma (1997) included ion hydration and dielectric saturation effects where the change in pore dielectric constant induced by the radial electric field was defined by the Booth equation.

Wang *et al.,* (1995a) compared the TMS model to the SCM and found the results to be in good agreement provided the pore radius was significantly smaller than the Debye length. In such a case, the electrical double layers formed within the pore overlap and the radial variation in concentration and potential is small. Bowen *et al.* (1997) showed this assumption of radial homogeneity to be satisfied by a wide range of NF conditions since surface charge density is reasonably small and pores are narrow. Therefore, at present, the most widely adopted models of NF are based on this approximation and so are effectively developments of the original model of Tsuru *et al.,* (1991). Many similar versions of this model have been proposed although it is perhaps the work of Rios *et al.*, (1996), Wang *et al.*, (1997), Combe *et al.*, (1997) and Bowen *et al.*, (1997) that have made the greatest

contributions in this field. More recent examples of this DSPM model being applied to describe solute transport through a nanofiltration include Almazan et al., (2015) who described the transport of glucose through a Desal DK and Desal DL membrane, Fadei et al. (2012) who successfully applied the DSPM-Dielectirc model to mono- and di-valent ion solutions and again by Dey et al. (2012) who applied the model to describe the transport of lactic acid through a nanofiltration membrane.

To summarise the UDSPM model is selected on the basis that the model considers more phenomena to describe the transport across the membrane (convective, electric and diffusive transport) than that the of the Spiegler Kedem model (based on irreversible thermodynamics) and the Solution-diffusion model (assuming no presence of pores). Researchers in the field of nanofiltratrion are in agreement that there are pores present and that the majority of transport is via pore flow. The term 'majority' is used to describe the transport as inevitably some transport would occur through the membrane polymer matrix via solution diffusion, however most of the transport would be via the pores present, i.e 'path of least resistance'.

4.1 Experimental determination of the hindrance factors for nanofiltration and subsequent evaluation of current theoretical models

4.1.1 Introduction

Membrane based processes for the separation and concentration of valuable compounds have received significant attention in the past three decades and the future remains promising with significant growth expected. The driving factors for growth in membrane technologies are attributed to the ease of operation and scale up, low operational cost, low energy requirement and high selectivity factors (Bringas et al., 2009). Nanofiltration (NF) is a pressure driven membrane separation technique situated between ultrafiltration and reverse osmosis. NF membranes are typically polymeric, asymmetric and consist of a low resistance support layer with a functionally active porous top layer (Schafer et al., 2005; Lau et al., 2012). The

nominal molecular weight cut-off of an NF membrane is in the range 100-1000 Da, indicating that the NF membrane active layer has an approximate pore size of 1 nm.

NF is an extremely complex process and is dependent on the micro-hydrodynamic and interfacial events occurring at the membrane surface and within the membrane nanopores. The nano-scale phenomena involved in neutral and charged solute separations by NF are extremely complex and, as such, are likely to be a rigorous test of any macroscopic description of ion transport and partitioning. The optimisation of NF membrane equipment along with an expansion in potential applications would be greatly facilitated if simple, accurate and quantitative methods for predicting process performance were available. An ideal method would combine the physical property data of a given process stream and a membrane along with a fundamental mathematical description of the transport processes so that the required optimum separation conditions could be determined. However, the near atomic length scales of the nanofiltration process leads to inherent problems when considering existing macroscopic descriptions of hydrodynamics and solute-pore interactions (Bowen and Mukhtar, 1996).

Solutes moving in free solution experience drag forces exerted by the solvent flowing through the confined pore structure. The movement of solutes in this confined space is greatly affected by the local environment and the transport of the solute is considered as hindered. Hindered transport can be expressed in terms of both a convective and diffusive element which contributes to the overall transport effect. Theories describing hindered transport have received attention since the mid 1900's, with most considering biological applications (Deen, 1987). This early work utilised equivalent pore analysis to describe lipid-insoluble permeation of capillary endothelial walls, red blood cells and the nuclear envelope of eukaryotic cells as well as artificial membranes in terms of movement in water filled pores (Paine and Scherr, 1975).

The effective diffusion coefficient of a solute within a pore of similar size is usually lower than that of the bulk solution due to the confinement (Davidson and Deen, 1988). Diffusion through pores is also hindered due to the irregular pore structure;

current descriptions of this phenomena assume a perfectly cylindrical pore, however, in reality the pore structure is most likely far from perfectly cylindrical. Restricted pore diffusion has been studied for decades and indicates that hindered diffusivity is smaller than that of the diffusivity of the free solution (Koh et al., 1998). The scale of nanopores would suggest that the description of diffusion is definitely hindered and is likely to deviate in a similar fashion to that of Knudsen diffusion. Knudsen diffusion considers the diffusion of a gas molecule through a small nano-scale pore, however, the spacial confinement of a nanopore would physically allow the transport of only single solutes and solvents at a time. The convective hindrance factor, K_c, describes the effects that flow of the solvent in the confines of the pore (including wall effects) has on the solute being transported and represents the ratio of solute velocity to solvent velocity in the pore. The diffusive hindrance factor, K_d, represents the ratio of the hindered diffusivity of the solute in the pore to that of the bulk solution. The values for these convective and diffusive drag forces in membrane pores are usually calculated from complex hydrodynamic flow equations using finite element techniques (Bowen and Sharif, 1994; Kim and Zydney, 2004; Silva et al., 2009). To the best knowledge of the authors there are no experimentally derived correlations relating real experimental data to hydrodynamic drag in cylindrical pores of nano-scale dimensions. Dechadilok and Deen (2006) listed a number of studies involving the microscopic visualization of the motion of individual particles in slit-like pores. Despite the obvious difference between a slit and an assumed cylindrical nano-pore, the scale of the solutes and pores listed are orders of magnitude greater than those found in a nanofiltration membrane separation.

The aim of this work is to experimentally determine the convective and diffusive hindrance factors associated with the transport of solutes in nanopores and then make a comparison of these experimentally derived hydrodynamic factors to those obtained by theoretical means. This will facilitate the validation of such theoretical descriptions for NF membrane processes and will confirm that the models in use are a true representation of the complex phenomena occurring in the real separation process. The experimental work will study transport performance of a

polymeric membrane with a series of polyethyleneglycols (PEGs) of differing molecular weight. PEG is a neutral solute which negates the charge separation mechanism of the NF membrane and significantly reduces the complexity of the Nernst-Planck equation simplifying the calculation of the hindrance factors from rejection data.

4.1.2 Relevant Theory

A solute moving in a solution experiences a resistance or drag force from the solvent. The magnitude of the drag force exerted on a particular solute is dependent on the shape and size of the solute as well as the viscosity of the solvent. In the case of a spherical solute in dilute bulk solution the drag force experienced may be expressed as:

$$F_{\infty} = (6\pi\eta r_s)u_s \qquad [4.1.1]$$

where F_{∞} is the drag force in the bulk solution, η is the solvent viscosity, r_s is the radius of the solute and u_s is the solute velocity. In the case where the solute is much larger than the solvent the Stokes-Einstein equation may be applied which relates the diffusion coefficient of the solute in the bulk solution, $D_{i,\infty}$, to that of the Stokes-Einstein radius of the solute, r_s (Deen, 1987):

$$r_s = \frac{kT}{6\pi\eta D_{i,\infty}} \qquad [4.1.2]$$

where $k$is the Boltzmann's constant and $T$is the absolute temperature.

Similar hydrodynamic constraints are applicable for the convective transport of a solute through pores, therefore eqns. [4.1.1] and [4.1.2] can be adapted to describe the drag force experienced by a solute when moving in a pore.

Hindrance (or drag) factors are introduced into the NF models to account for the hindered passage of solutes through the confined polymer structure of the NF membrane. The hindrance factors for movement inside an interconnecting network of polymers are difficult to derive and have not been reported so far. Therefore, all of the work in this area to date has assumed a solute of rigid spherical

shape moving through a perfectly cylindrical pore of infinite length as shown in Figure 4.1.1.

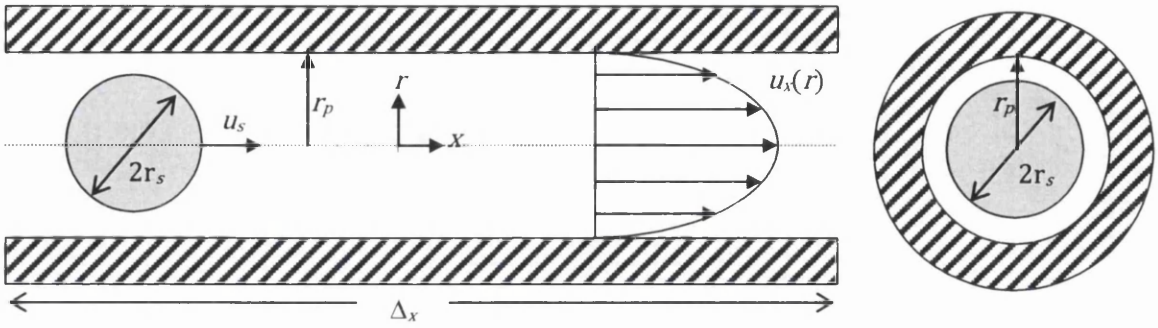

Figure 4.1.1: Schematic diagram of the movement of a rigid spherical solute inside a perfectly cylindrical pore

The solute with radius r_s is moving with velocity u_s in the direction x along the centreline of the cylindrical pore of radius r_p and length Δx. For such a case, expressions for the hindrance factors can be derived theoretically from a fundamental knowledge of the system hydrodynamics (Deen, 1987). The derivation of hindrance factor expressions require the following basic assumptions:

I. The radius of the pore r_p and solute molecule r_s greatly exceed that of the solvent.
II. Pore flow is fully developed with a very low Reynolds number one pore radius downstream of the pore entrance.
III. Mass transfer effects are neglected at the pore entrance and exit.
IV. The bulk solution is assumed to be sufficiently dilute to minimise all solute-solute interactions.

When the flow is assumed to be at an isothermal condition, the drag force experienced by the solute is exactly balanced by hydrodynamic forces to give:

$$-kT\,\frac{\delta \ln c}{\delta x} - 6\pi\eta r_s\,K(u_s - Gu_x) = 0 \qquad [4.1.3]$$

where u_x is the unperturbed fluid velocity upstream or downstream of the particle, c is the concentration, K is the hydrodynamic coefficient and G is the lag

coefficient, accounting for the effects of the finite pore size. In an unbounded fluid, $K = G = 1$ and the second term is equivalent to Stokes' Law (eqn. 4.1.1). The left hand term of eqn. [4.1.3] represents the diffusional force per solute in the x direction and the right hand term corresponds to the hydrodynamic forces.

Defining solute flux as $J_s = u_s c$ allows eqn. [4.1.3] to be rearranged resulting in:

$$J_s = -K^{-1} D_\infty \frac{\delta c}{\delta x} + G u_x c \qquad [4.1.4]$$

Eqn. (4.1.4) is the standard flux expression for a dilute liquid solution and accounts for the contribution of convection and diffusion modified by the factors G (a lag coefficient) and K^{-1} (an enhanced drag coefficient) respectively. These drag coefficients are dependent on the ratio of solute radius, r_s, to the pore radius, r_p, usually expressed as:

$$\lambda = \frac{r_s}{r_p} \qquad [4.1.5]$$

The assumption of a long cylindrical pore results in the unperturbed fluid velocity having a parabolic profile given as:

$$u_x = 2\hat{u}_x(1 - \beta^2) \qquad [4.1.6]$$

where $\hat{u}_x$ is the average solvent velocity over the pore cross section and $\beta = \frac{r}{r_p}$ (a dimensionless radial position). The subsequent average solute flux $\hat{J}_s$ over the pore cross section is expressed as:

$$\hat{J}_s = \frac{\int_0^1 J_s \beta d\beta}{\int_0^1 \beta d\beta} = 2\int_0^{1-\lambda} J_s \beta d\beta \qquad [4.1.7]$$

Substitution of eqn. [4.1.4] into eqn. [4.1.7]results in:

$$\hat{J}_s = -2D_\infty \int_0^{1-\lambda} K^{-1} \frac{\delta c}{\delta x} \beta d\beta + 4\hat{u}_x \int_0^{1-\lambda} Gc(1-\beta^2)\beta d\beta \qquad [4.1.8]$$

The concentration term, c, may be approximated by:

$$c = g(x)\exp^{-\frac{E(\beta)}{kT}} \qquad [4.1.9]$$

where $g(x)$is the function describing the variation in axial concentrations within the pore and E is the term describing the electrostatic interactions. Combining eqns. [4.1.8] and [4.1.9] results in the final local flux equation, given as:

$$\hat{J}_s = -K_d D_\infty \frac{d\hat{c}}{dx} + K_c \hat{u}_x \hat{c} \qquad [4.1.10]$$

where K_d and K_c are the integral of inverse enhanced drag and the lag coefficient respectively.

K_d is then described as:

$$K_d = \frac{\int_0^{1-\lambda} K^{-1} exp^{-E/kT}\, \beta d\beta}{\int_0^{1-\lambda} exp^{-E/kT}\, \beta d\beta} \qquad [4.1.11]$$

and K_c is defined as:

$$K_c = \frac{2\int_0^{1-\lambda} G(1-\beta^2)\, exp^{-E/kT}\, \beta d\beta}{\int_0^{1-\lambda} exp^{-E/kT}\, \beta d\beta} \qquad [4.1.12]$$

The partitioning coefficient (ratio of the average intrapore concentration to that of the bulk solution), ϕ, was defined by Deen (1987) for the boundary conditions $x = 0, g(0) = C_o$ and when $X = \Delta x, g(L) = C_{\Delta x}$as:

$$\phi = \frac{\hat{C}_0}{C_0} = \frac{\hat{C}_L}{C_L} = 2\int_0^{1-\lambda} exp^{-E/kT}\, \beta d\beta \qquad [4.1.13]$$

where C_0 and C_L are the solute concentrations in the external solution adjacent to the membrane surface. However in the case of a neutral solute and for purely steric interactions between the solute and the pore wall ($E = 0$), the partitioning coefficient then reduces to:

$$\phi = (1 - \lambda)^2 \qquad [4.1.14]$$

The integration of eqn. [4.1.11] with the same boundary conditions of eqn. [4.1.13] leads to the macroscopic flux equation:

$$\hat{J}_s = W\, \hat{u}_x C_0 \frac{[1-(C_L/C_0)exp^{-Pe}]}{[1-exp^{-Pe}]} \qquad [4.1.15]$$

where W is defined as:

$$W = \phi K_c = 4 \int_0^{1-\lambda} G(1-\beta^2) exp^{-E/kT} \beta d\beta \quad [4.1.16]$$

and Pe is the Peclet number based on pore length described as:

$$Pe = \frac{W \hat{u}_x \Delta x}{H D_\infty} \quad [4.1.17]$$

with H defined as:

$$H = \phi K_d = 2 \int_0^{1-\lambda} K^{-1} exp^{-E/kT} \beta d\beta \quad [4.1.18]$$

In the case of neutral solutes $E = 0$. Rearranging eqns. [4.1.16] and [4.1.17] for K_c and K_d respectively for cylindrical pores gives:

$$K_c(\lambda) = \frac{4}{(1-\lambda)^2} \int_0^{1-\lambda} G(\lambda, \beta)(1-\beta^2) \beta d\beta \quad [4.1.19]$$

$$K_d(\lambda) = \frac{2}{(1-\lambda)^2} \int_0^{1-\lambda} \frac{\beta d\beta}{K(\lambda,\beta)} \quad [4.1.20]$$

The limits of eqn. (4.1.15) are as follows:

$$\hat{J}_s = \frac{H D_\infty}{\Delta x} (C_0 - C_{\Delta x}) \qquad (Pe < 1) \quad [4.1.21]$$

$$\hat{J}_s = W \hat{u}_x C_0 \qquad (P > 1) \quad [4.1.22]$$

where solute transport is dominated by diffusion when $Pe < 1$ and by convection when $Pe > 1$.

Studies have demonstrated that a centreline approximation for the solute location in the nanopore provides an accurate estimation of the hydrodynamic coefficients (Bowen and Mukhtar, 1996). Thus, for an uncharged interaction where $E = 0$, a Hagen-Poiseuille velocity profile and the centreline approximation results in eqn. [4.1.16] and [4.1.18] becoming:

$$K_{i,c} = \frac{u_s}{u_x} = (2 - \phi_i) G(\lambda, 0) \quad [4.1.23]$$

$$K_{i,d} = \frac{D_{i,p}}{D_{i,\infty}} = K^{-1}(\lambda, 0) \quad [4.1.24]$$

where $D_{i,p}$ is the hindered diffusivity inside the NF pore, u_s is the solute velocity and u_x is the maximum solvent velocity.

The pore radius of an NF membrane is very small and as a direct consequence the solute velocity profile may not be fully developed. In such a case where a homogeneous velocity profile is more appropriate $K_{i,c}$ reduces to (Bowen et al., 1997):

$$K_{i,c} = G(\lambda, 0) \qquad [4.1.25]$$

A review of the theoretical calculations for the determination of the hindrance factors has been conducted [Deen (1987), Dechadilok and Deen (2006)] and point value solutions calculated using finite element methods are available for the hindrance factors, provided in Table 4.1.1.

Table 4.1.1: Point values for hindrance factors determined by finite element methods. The values reported for $\lambda \leq 0.4$ are the original data from Anderson and Quinn (1974), and the additional values are obtained from Oatley (2004).

λ	K^{-1}	G	Φ	$K_{i,c}$	$K_{i,d}$
0	1	1	1	1	1
0.05	0.8950	0.9983	0.9025	1.09565	0.89504
0.10	0.7916	0.9932	0.8100	1.18187	0.79164
0.20	0.5955	0.9720	0.6400	1.32196	0.59553
0.30	0.4228	0.9356	0.4900	1.41275	0.42278
0.40	0.2822	0.8829	0.3600	1.44796	0.28223
0.50	0.1673	0.8343	0.2500	1.46009	0.16734
0.60	0.0892	0.7660	0.1600	1.40946	0.08918
0.70	0.0407	0.7081	0.0900	1.35256	0.04067
0.80	0.0134	0.6379	0.0400	1.25032	0.01345
0.90	0.0021	0.5676	0.0100	1.12951	0.00213
0.92	0.0012	0.5540	0.0064	1.10447	0.00119
0.95	0.0004	0.5335	0.0025	1.06561	0.00035
0.98	0.0000	0.5128	0.0004	1.02531	0.00003

Several authors have fitted polynomial expressions to these point values and these are summarised, along with the valid range, in Table 4.1.2. Figure 4.1.2 illustrates these point values for hindrance factors and demonstrates the polynomial fitting expression of Oatley (2004) as an example. Table 4.1.2 indicates that there are many alternative expressions derived for the simple calculation of the hindrance

factors. These models all assume centreline positioning of the particle, however, the model produced by Dechadilok and Deen (2006) averaged the properties of the convective and diffusive forces across the pore cross section for cylindrical pores.

Table 4.1.2: Theoretical descriptions for the convective (K_c) and diffusive (K_d) hydrodynamic coefficients

Model	Equation	Range	Reference
Anderson and Quinn	$K_d = 1 - 2.1044\lambda + 2.089\lambda^3 - 0.948\lambda^5$ $K_c = (1 + 2\lambda - \lambda^2)(1 - (2/3)\lambda^2 - 0.163\lambda^3)$	$0 < \lambda \leq 0.4$	Anderson and Quinn (1972)
Brenner and Gaydos	$K_d = \frac{1 - (9/8)\lambda \ln \lambda^{-1} - 1.539\lambda}{1 - 2\lambda + \lambda^2}$ $K_c = (1 + 2\lambda - 4.9\lambda^2)$	$0 < \lambda \leq 0.1$	Brenner and Gaydos (1977)
Bowen and Sharif	$K_d = -1.705\lambda + 0.946$ $K_c = (-0.301\lambda + 1.022)(1 + 2\lambda - \lambda^2)$	$0 < \lambda \leq 0.4$	Bowen and Sharif (1994)
Bowen et al.	$K_d = 1 - 2.3\lambda + 1.154\lambda^2 + 0.224\lambda^3$ $K_c = (1 + 0.054\lambda - 0.988\lambda^2 + 0.441\lambda^3)(1 + 2\lambda - \lambda^2)$	$0 < \lambda \leq 0.8$	Bowen et al. (1997)
Bandini and Vezanni	$K_d = -0.105 + 0.318\lambda - 0.213\lambda^2$ $K_c = (-6.830 + 19.348\lambda - 12.518\lambda^2)(1 + 2\lambda - \lambda^2)$	$0.8 < \lambda \leq 1$	Bandini and Vezanni (2003)
Mavrovouniotis and Brenner	$K_d = \frac{0.984(1 - \lambda)^{9/2}}{1 - 2\lambda + \lambda^2}$	$\lambda > 0.9$	Mavrovouniotis and Brenner (1986) adapted from Silva et al. (2009)
Haberman and Sayre	$K_d = \frac{1 - 2.105\lambda + 2.0865\lambda^3 - 1.7068\lambda^5 + 0.7260\lambda^6}{1 - 0.75857\lambda^5}$ $K_c = \frac{(1 + 2\lambda - \lambda^2)(1 - (2/3)\lambda^2 - 0.20217\lambda^5)}{1 - 0.75857\lambda^5}$	$0 < \lambda \leq 0.9$	Haberman and Sayer (1958)
Oatley	$K_d = 1 - 2.1812\lambda + 0.7328\lambda^2 - 0.9065\lambda^3 + 6.7272\lambda^4 - 10.2324\lambda^5 + 6.3293\lambda^6 - 1.4692\lambda^7$ $K_c = (1 + 2\lambda - \lambda^2)(1 + 0.0650\lambda - 1.9370\lambda^2 + 8.5211\lambda^3 - 27.3398\lambda^4 + 44.4150\lambda^5 - 34.4150\lambda^6 + 10.3358\lambda^7)$	$0 < \lambda \leq 0.98$	Oatley (2004)
Dechadilok and Deen	$K_d = \frac{1}{1 - \lambda^2}\left(1 + \frac{9}{8}\lambda \ln\lambda - 1.5603\lambda + 0.52815\lambda^2 + 1.9152\lambda^3 - 2.8190\lambda^4 + 0.27078\lambda^5 + 1.10115\lambda^6 - 0.43593\lambda^7\right)$ $K_c = \frac{1 + 3.867\lambda - 1.907\lambda^2 - 0.834\lambda^3}{1 + 1.867\lambda - 0.741\lambda^2}$	$0 < \lambda \leq 0.95$	Dechadilok and Deen (2006)
Bungay and Brenner	$K_d = \frac{6\pi}{K_t} \quad K_c = (1 + 2\lambda - \lambda^2)\frac{K_s}{2K_t}$ $K_t = \frac{9}{4}\pi^2\sqrt{2}(1 - \lambda_i)^{-5/2}\left(1 + \sum_{n=1}^{2} a_n (1 - \lambda_i)^n\right) + \sum_{n=0}^{4} a_{n+3}\lambda_i^n$ $K_s = \frac{9}{4}\pi^2\sqrt{2}(1 - \lambda_i)^{-5/2}\left(1 + \sum_{n=1}^{2} b_n (1 - \lambda_i)^n\right) + \sum_{n=0}^{4} b_{n+3}\lambda_i^n$	$0 < \lambda \leq 1$	Bungay and Brenner (1973)

a_1	a_2	a_3	a_4	a_5	a_6	a_7
-1.2167	1.5336	-22.5083	-5.6117	-0.3363	-1.216	1.647
b_1	b_2	b_3	b_4	b_5	b_6	b_7
0.1167	-0.0442	4.018	-3.9788	-1.9215	4.392	5.006

Silva et al. (2009) produced similar plots to that in Figure 4.1.2 but extrapolated beyond the limits of validity for many of the proposed expressions. Essentially, any of these expressions may be used to calculate the magnitude of the hindrance factors so long as the calculation and model in use are within the determined valid range. The authors would recommend the model proposed by Oatley (2004) as this spans the maximum range of λ, particularly important when modelling NF pore size distributions.

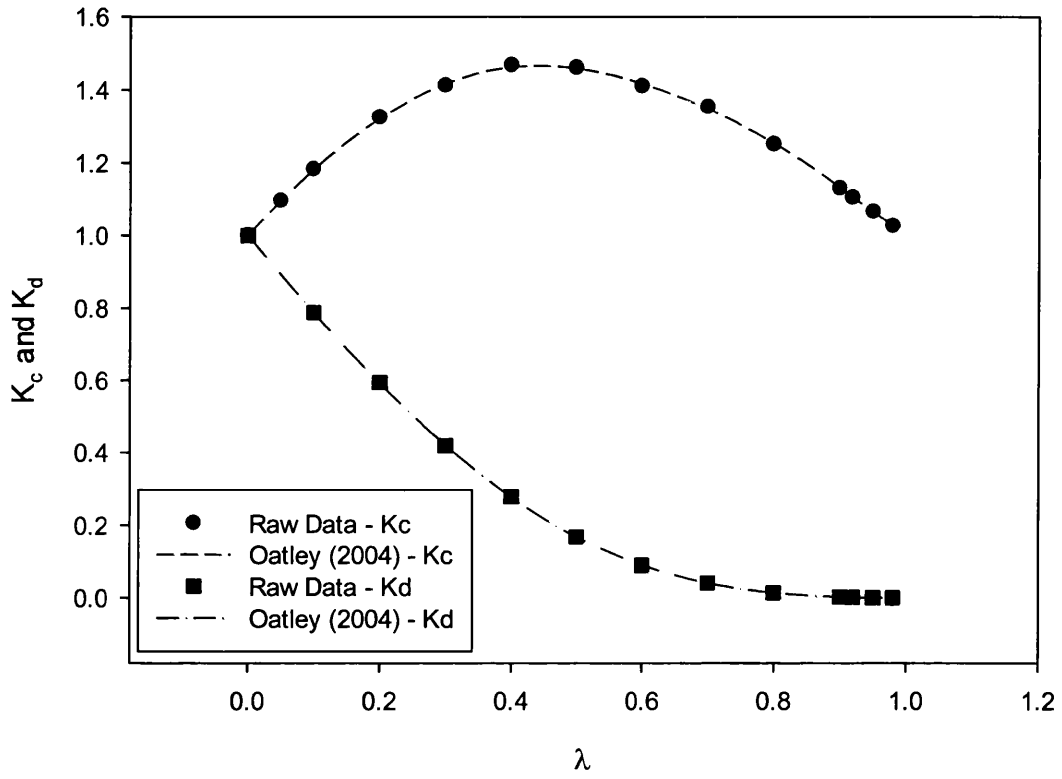

Figure 4.1.2: Raw data for diffusive and convective pore hindrance factors proposed in the literature along with the polynomial fitting of Oatley (2004) as an example

The transport of ions through a nanofiltration membrane is typically described using the extended Nernst-Planck equation:

$$j_i = -\frac{c_i K_{i,d} D_{i,\infty}}{RT}\frac{d\mu}{dx} + K_{i,c} c_i V \qquad [4.1.26]$$

where j_i is the ionic flux, c is the concentration, V is the solvent velocity and $K_{i,c}$ and $K_{i,d}$ are the hindrance factors to account for the convection and diffusion in the confined NF pore.

For a neutral species, the solution of eqn. [4.1.26] is much simplified and leads to an analytical expression to calculate rejection as [Bowen and Welfoot, 2002; Oatley-Radcliffe et al., 2014]:

$$R = 1 - \frac{C_{i,p}}{C_{i,w}} = 1 - \frac{K_{i,c}\phi_i}{1-[1-\phi_i K_{i,c}]exp(-Pe')} \quad [4.1.27]$$

where $Pe' = \frac{K_{i,c}}{K_{i,d}} \frac{r_p^2}{8\eta D_{i,\infty}} \Delta P_e$ is a modified Peclet number and ΔP_e is the effective pressure driving force ($\Delta P - \Delta\Pi$). At limiting rejection, where $\Delta P_e \rightarrow \infty$, this expression simplifies further to yield:

$$R_i = 1 - K_{i,c}\phi_i \quad [4.1.28]$$

The rejection in eqn. [4.1.28] is dependent only upon λ and the hindrance coefficient $K_{i,c}$. Therefore, from a knowledge of the membrane pore size, the solute size and the experimental limiting rejection the hindrance factor $K_{i,c}$ may be calculated. Then, with the values mentioned previously, and experimental rejection determined over a range of pressure $K_{i,d}$ can be calculated.

4.1.3 Materials and Methods

The experimentation undertaken in this section was undertaken as described in Sections 3.1 and 3.2.3. All rejection experiments were carried out at laboratory scale using a frontal filtration set-up as described in Figure 3.3. For this experimental work only the Membranology cell (Membranology Ltd., Swansea, UK) was used and the equipment properties are described in chapter 3.

4.1.4 Results & Discussion

Particle Sizing

The values for particle size obtained from the zetasizer were compared to current models and literature values for various small solutes in order to gain an understanding of the accuracy and validity of the equipment and current predictive models for particle size.

The predictive models used in this study were those of:

Combe et al (1999), derived to predict the diffusion coefficients of various forms of PEG:

$$r_s = 0.045MW^{0.44} \quad [4.1.29]$$

Singh et al (1998), developed from intrinsic viscosity data:

$$r_{si} = 16.73 \times 10^{-10} MW^{0.557} \quad [4.1.30]$$

Bowen and Mohammad (1998), developed to predict small molecule diffusivity:

$$\log_{10} r_s = -1.3363 + 0.395 \log_{10} MW \quad [4.1.31]$$

The experimental findings and their comparison to other literature experimentation and values obtained from the predictive models are illustrated in Table 4.1.3.

Table 4.1.3: Measured sizing data for PEG from the Zetasizer compared with other experimental and theoretical values. *values from this study

	Measurements			Theoretical	
	DLS Measurements		PEG diffusion measurements	Stokes-Einstein of PEGs	Known values of small solutes
Molecular Weight (Da)	**Zetasizer* (r.nm)**	**Oatley (2004) (r.nm)**	**Combe et al. (1990) (r.nm)**	**Singh et al. (1998) (r.nm)**	**Bowen and Mohammad (1998) (r.nm)**
200	0.339	-	0.463	0.32	0.374
400	0.483	-	0.628	0.471	0.491
600	0.584	-	0.751	0.59	0.577
1000	0.726	-	0.94	0.784	0.706
1450	0.814	1.11	1.107	0.965	0.817
2000	1.062	-	1.275	1.154	0.928
3400	1.149	1.75	1.611	1.551	1.145
4600	1.299	1.85	1.84	1.835	1.290

The size data obtained using the zetasizer measurements in this study are similar to those obtained by Singh et al. (1998) and Bowen and Mohammad (1998) for molecular weights below 1000, thus suggesting that the zetasizer is providing accurate sizing data within this range. However there is a significant disparity between the data obtained by Combe et al. (1990) and the zetasizer values obtained in this study. The experimental data was then compared to the predictive models and was most accurately predicted by the model of Bowen and Mohammad (1998) over the whole range of PEGs studied. For example the average difference between the experimentally derived values in this work and the values obtained by Coombe et al. (1990), Singh et al. (1998) and Bowen and Mohammad (1998) is 28.1, 13.3 and 5.4% respectively. The largest discrepancy is seen for PEG 4600 where the experimentally derived radius is 1.299 nm whereas the values quoted by Oatley (2004), Coombe et al. (1990) and Singh et al. (1998) are all greater than 1.8 nm, while Bowen and Mohammad report a particle radius of 1.145 nm. Interestingly, the PEG measurements by Oatley (2004) were performed on a HPPS (High Performance Particle Sizer) a predecessor of the current zetasizer equipment. As the trend of the experimental data is sensible and the vast majority of the data values concur with other values reported elsewhere in the literature, the experimentally determined values for PEG sizes were deemed acceptable and used in all subsequent calculations.

Membrane Pore Size

The accurate measurement of pore sizes in nanofiltration membranes is an area of contention, although a variety of methods have been used to measure the membrane pore size. These include AFM (Hilal et al., 2004), neutral solute rejection (Garcia-Martin et al., 2014), liquid-liquid displacement (Otero et al., 2008) and BET (Fang et al., 2014). This study used three different independent measurement methods in order to estimate the membrane pore size, namely AFM, BET and neutral solute rejection. The membrane used in this study is reported by the manufacturer to have a molecular weight cut-off (MWCO) of 4000, although due to differing determination methods for each manufacturer the calculation of membrane separation characteristics is not accurate from MWCO alone.

Neutral Solute Rejection: The experimental approach undertaken allowed the estimation of membrane MWCO using the rejection data obtained for the PEGs studied. Membrane MWCO is often estimated using the molecular weight at which 90% of the solute is rejected.

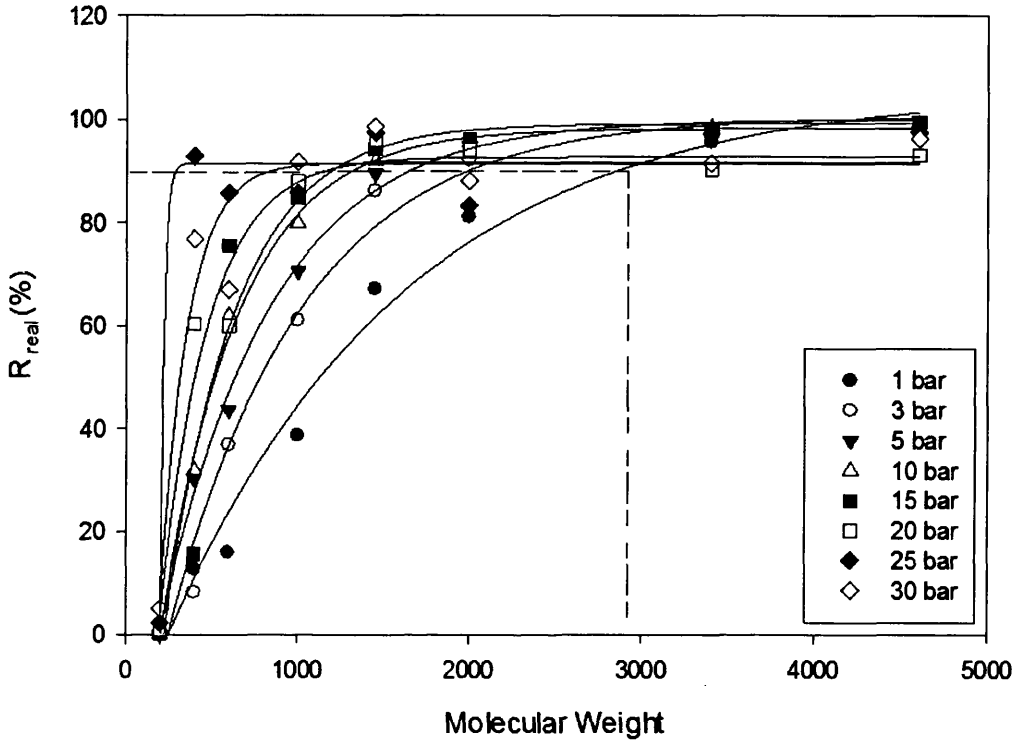

Figure 4.1.3: PEG rejection with varying molecular weight from the Nadir UH004 membrane

Figure 4.1.3 represents the real rejection of the PEGs studied plotted against molecular weight. The lines plotted on the figure represent the fitting of the exponential rise to maximum expression [eqn. 3.11]. Figure 4.1.3 shows that at 2900 Da more than 90 % of the solute is rejected at all pressures studied suggesting that the 4000 MWCO claimed by the manufactures is an overestimation. Using the particle size correlations eqns. [4.1.29] and [4.1.31] and assuming the presence of perfectly cylindrical pores, a MWCO of 2900 (90% rejection value) gives a pore diameter of 3.00 and 2.06 nm respectively. The data shown in Figure 4.1.3 shows that with increasing pressure the apparent MWCO decreases, illustrating that a quoted MWCO is highly dependent on the characterisation conditions used.

AFM measurements: A topographic AFM image of the Nadir UH004 membrane is shown in Figure 4.1.4. The membrane has a relatively low roughness (scan size 0.5 μm^2) of 0.4 ± 0.012 nm. The data obtained from the AFM measurements suggest an average pore radius of 1.29 nm, with a minimum pore size measurement of 0.55 nm and a maximum measured pore size of 2.02 nm. The darker shaded areas in Figure 4.1.4 are the areas where pores are present. The membrane porosity is calculated as 2.48%.

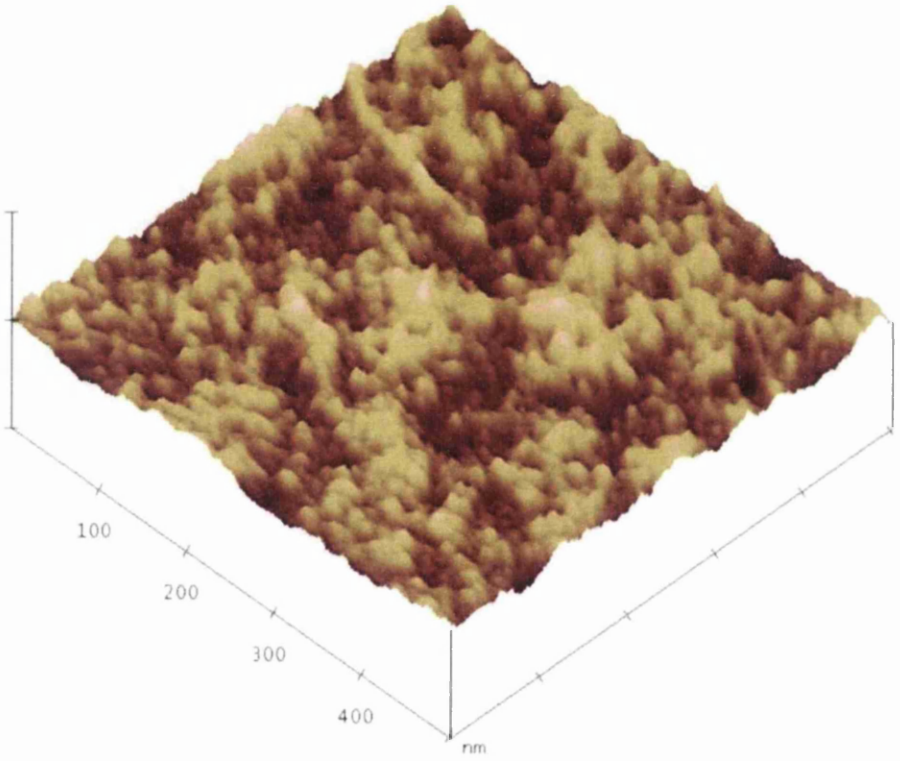

Figure 4.1.4: Topographic AFM image of the Nadir 4000 MWCO membrane

BET measurement: The nitrogen adsorption-desorption isotherm of the membrane studied is illustrated in Figure 4.1.5a. Application of the HK method for pore-size analysis suggests a membrane pore radius of 0.86 nm and is in the expected range for a membrane at the loose end of NF. Furthermore the pore size obtained is similar to the pore-size obtained by the AFM measurement. The pore size distribution obtained from the nitrogen adsorption-desorption isotherm is shown in Figure 4.1.5b and suggests that there are no pores larger than 0.97 nm and no pores smaller than 0.3 nm. The isotherm obtained in this experimental work shows a slight disparity between the adsorption and desorption isotherms. This

discrepancy was observed in all experimental BET runs undertaken regardless of the mass of membrane placed in the equipment and the phenomenon is attributed to the effect that the membrane support layer has on the adsorption/desorption performance of the BET machine. The difference in pore size distribution determined using the BET method from the AFM method is attributed to natural variance expected between the two methodologies for measurements at nanometre length scales.

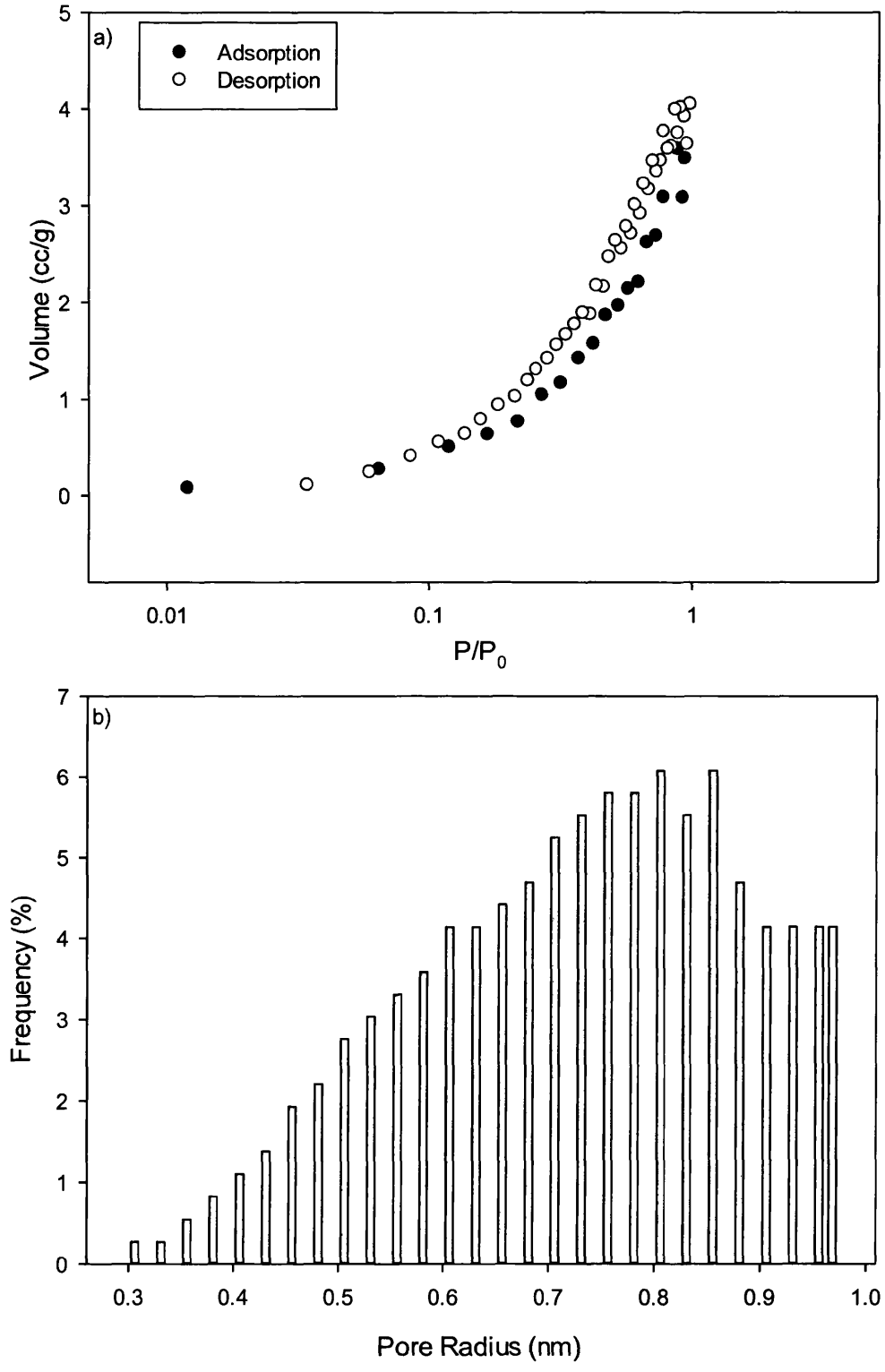

Figure 4.1.5: a) BET Nitrogen adsorption/desorption isotherm for the Nadir UH004 membrane, b) BET pore size distribution

The data obtained from the independent pore size measurements suggest a pore size diameter in the region of 0.3 – 2.0 nm. For this reason a pore size of 1.075 nm, the average of the mean pore size from each method, will be used for all further calculations.

Rejections and hindrance factors

The observed rejection plotted against applied pressure for the various PEG solutions is shown in Figure 4.1.6a. The data obtained shows that PEG rejection increases with increasing PEG molecular weight as would be expected. For example, PEG 200 shows almost zero observed rejection, i.e. PEG fully transported across membrane, while PEG 4600 shows almost total rejection (100 %). However, in all cases, as the applied pressure increases beyond ~10 bar the PEG rejection dramatically decreases. For instance PEG 3400 displays a rejection of ~90% at low pressure and at 10 bar begins to fall to a minimum observed rejection of ~10% at 30 bar. This reduction in observed rejection is a result of concentration polarisation and is increasing with increasing applied pressure leading to higher membrane flux (Oatley et al., 2012). Figure 4.1.6b shows the real rejection (calculated using eqn. 3.5) plotted against applied pressure. The lines plotted on Figure 4.1.6b are the statistical regression of the data using eqn. [3.11] allowing the determination of the limiting rejection. Similarly to Figure 4.1.6a, PEG rejection increases with increasing molecular weight. PEG 200 is seen to have the minimal rejection of all the PEGs studied as expected and reaches a maximum rejection of ~5% at 30 bar. The effect of increasing pressure is seen to increase rejection in the majority of PEGs studied; this effect is particularly profound in PEGs 400, 600, 1000, 1450, 2000 and sucrose. PEGs 3400 and 4600 display almost constant rejection over the total pressure range studied with all rejections above 90%. Figure 4.1.6b indicates that the lower molecular weight species, PEG 200, PEG 400 and sucrose, do not achieve limiting rejection over the pressure range studied. So in the case of these three species, the limiting rejection was taken as the real rejection at the highest applied pressure. The result observed for PEG 400 is particularly strange in that the real rejection is seen to be above that of PEG 600 suggesting that some of the results obtained for PEG 400 are anomalous. The statistical curve fitting for all other solutes measured

suggests that the rejection has reached the limiting value and this maximum value was then used in subsequent calculations.

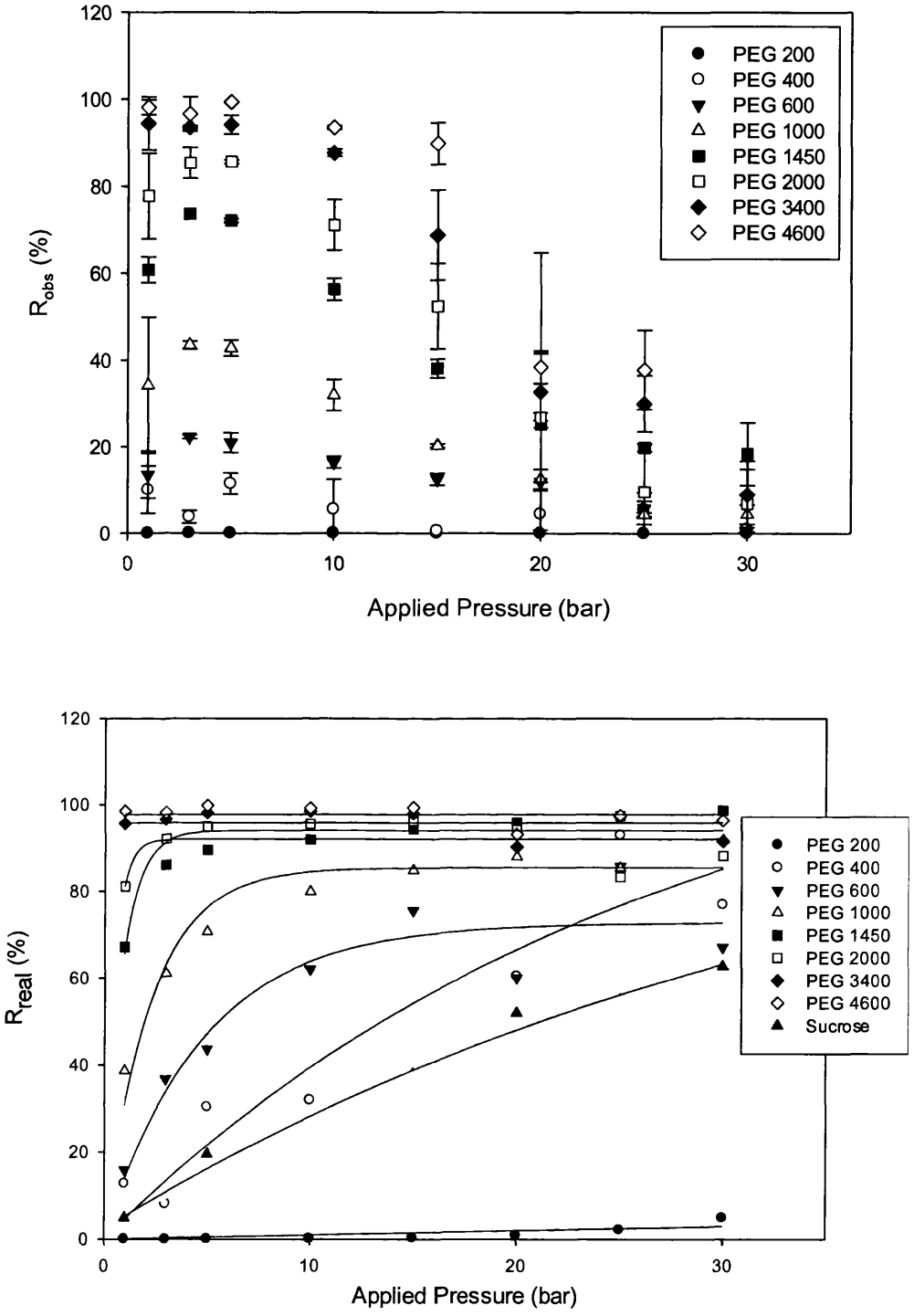

Figure 4.1.6: PEG rejection from the Nadir UH004 membrane with applied pressure (a) observed rejection and (b) real rejection

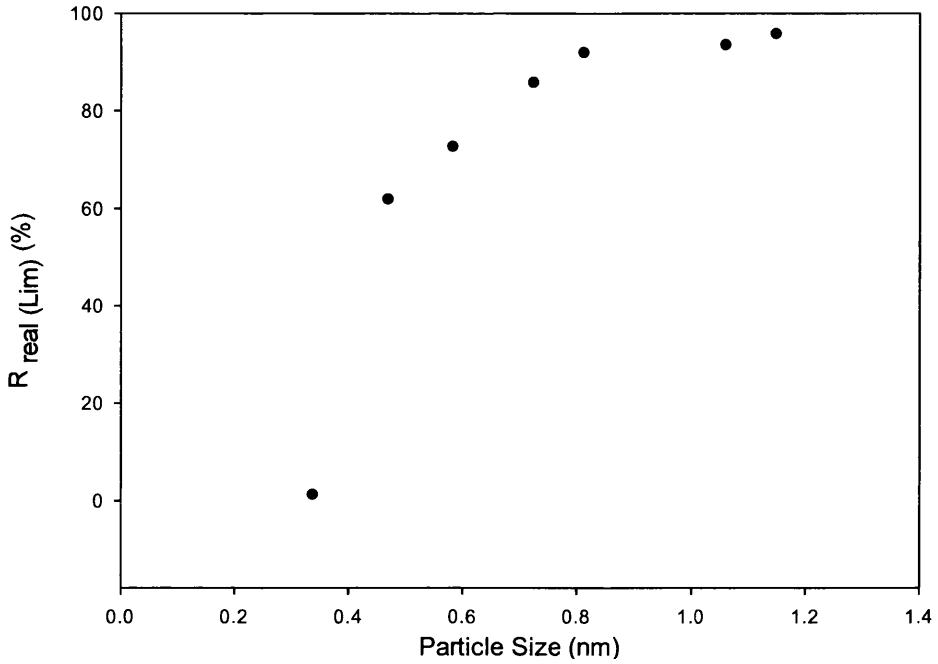

Figure 4.1.7: Limiting real rejection against measured particle size (sucrose data taken from Bowen and Mohammad, 1998)

The data points for the limiting rejection were then plotted against measured particle size and are illustrated as Figure 4.1.7. The limiting rejection increases with increasing particle size and rises to a maximum as expected. The values from Figure 4.1.7, along with eqn. [4.1.28], were then used to calculate the convective hindrance factors K_c and these are shown in Figure 4.1.8. The experimentally determined values for Kc from the PEG 400, 600, 1000 and 1450 are in close agreement to the theoretical data (Oatley, 2004). The Kc value obtained for PEG 200 deviates away from the theoretical prediction and this is attributed to the fact that limiting rejection has not been achieved for this species. A sensitivity analysis conducted into the magnitude of the limiting rejection value used for these species showed that a reduction of pore radius to 0.8 nm results in the calculated Kc values to increase and in some cases more than double, creating a significant disparity between the experimentally derived and theoretical values. An increase in pore radius to 1.2 nm results in the experimentally derived Kc values decreasing to below the theoretical line.

Once the convective hindrance factors were obtained, the diffusive hindrance factors could then be calculated from the rejection profile over the range of pressure using eqn. [4.1.27] and the derived values for pore size and particle size. The experimental value for the diffusive hindrance factor is also plotted in Figure 4.1.8.

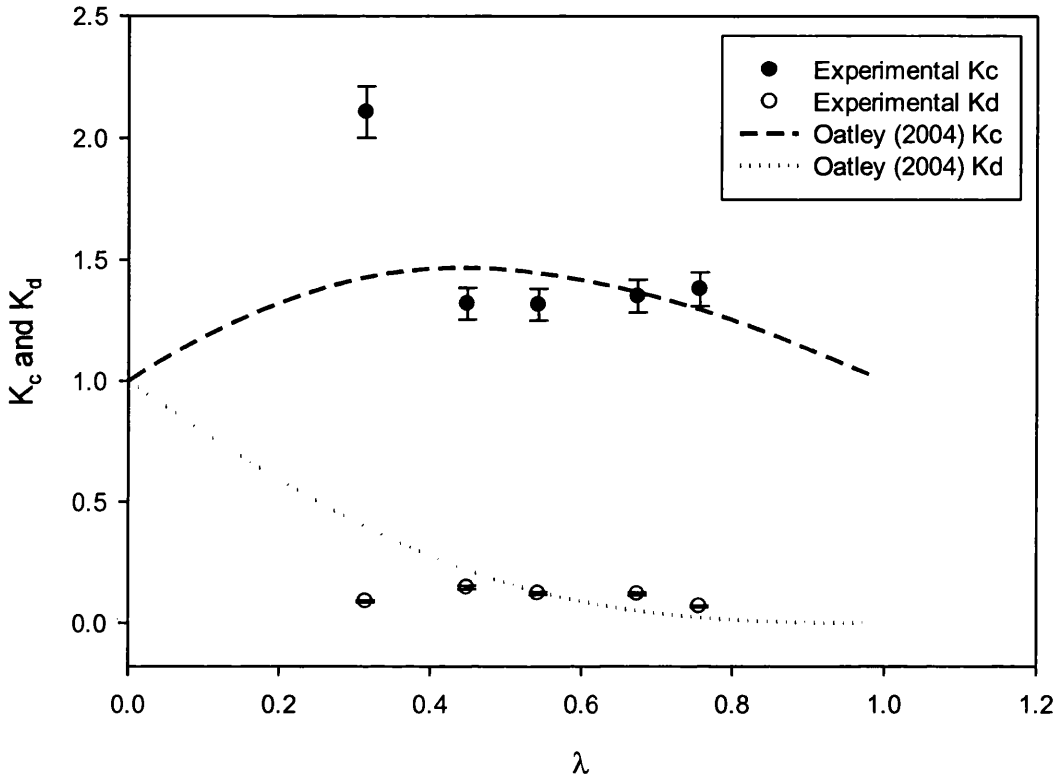

Figure 4.1.8: Experimentally derived hindrance factors (pore radius: 1.075 nm)

As for the convective hindrance factors, the results obtained for the diffusive hindrance factors are similar in magnitude to those values obtained from the finite element methods. The values do deviate away from the theoretically predicted line and exhibit a pseudo linear relationship. Interestingly, performing the same sensitivity analysis by varying the pore size in a similar fashion to that used previously for the convective hindrance factors results in very little variation of the K_d value. When considering the assumptions included in the development of hydrodynamic descriptions and the inherent experimental errors involved in this work, these experimental results provide reassurance in the validity of the

theoretical descriptions of these complex hydrodynamic phenomena and suggest that the currently accepted models derived for microfiltration and ultrafiltration do provide an accurate prediction of the hindrance factors and are suitable for use in NF applications.

4.1.5 Conclusions

This study has successfully used the rejection experiments of neutral solutes to determine the hindrance factors involved in the transport of materials through an NF membrane. To the best of the author's knowledge the experimental determination of such hydrodynamic forces for a nano scale membrane has not been conducted previously. The work confirms that the hydrodynamic forces are highly dependent on the ratio of solute to pore size. Three independent methods (neutral solute rejection, AFM and BET) were used to determine the pore size of the Nadir UH004 membrane and the average pore size was taken to be 1.075 nm. The particle size for a series of PEGs was independently determined using dynamic light scattering. This information along with a series of rejection profiles was used then used to experimentally determine both the convective and diffusive hindrance factors. The values obtained were deemed sensible and were compared to calculated point values obtained from hydrodynamic theory using finite element methods. Both sets of data were similar and the experimental values for hindrance factors were shown to be a fair representation of the theoretical data. This suggests that the correlations found throughout the literature are accurate enough for the calculation of the hindrance factors describing the hydrodynamic forces experienced by a solute in a nanopore.

4.2 Critical appraisal of current nanofiltration modelling and further insights on dielectric exclusion

The work contained in this sub-section attempts to further the understanding of nanofiltration modelling by studying the effects of dielectric exclusion. The work isolates the dielectric effect by operating at the membranes isoelectric point i.e. the point at which the membrane exhibits a net charge of zero. This allows the charge effects of the membrane to be discounted and the membrane dielectric effect to be evaluated separately. The effects of membrane charge on separation are well understood, however the effects of dielectric exclusion is less well understood and requires further investigation in order to improve understanding of the nanofiltration separation mechanisms.

4.2.1 Introduction

NF is an extremely complex process and is dependent on the micro-hydrodynamic and interfacial events occurring at the membrane surface and within the membrane nanopores. The nano-scale phenomena involved in uncharged solute and salt separations by NF are extremely complex and, as such, likely to be a rigorous test of any macroscopic description of ion transport and partitioning. The transport of uncharged solutes is reasonably well established through numerous studies of UF membranes. There have been many works over the past thirty years on modelling the transport of charged solutes across a charged membrane. A large number of predictive NF models have been based on either the charged capillary model (Jacazio et al., 1972), models based on the extended Nernst-Planck equation (Tsuru et al., 1991) or the irreversible thermodynamic model (Levenstein et al., 1996). The application of the extended Nernst-Planck equation was originally proposed by Schlogl (1966) for the description of transport of electrolytes in RO through ion-exchange membranes and is arguably the most commonly used model of modern times. The equation is particularly useful for NF as consideration is given to the mechanisms of transport and the adjustable fitting parameters required are based upon real measurable membrane properties. Rejection from NF membranes may be attributed to a combination of both steric and non-steric effects. The fact that the dimensions of the NF active layer are near atomic scale lengths, coupled with

limitations in current measurement technologies, has delayed a detailed knowledge of the physical structure and electrical properties of real NF membranes and has resulted in uncertainty and significant debate over the true nature of the separation mechanisms (Schafer et al., 2005) and the role of dielectric exclusion is particularly contested (Oatley et al., 2012).

The work contained in this section investigates the phenomena of dielectric exclusion and will show that our current understanding needs attention by considering the rejection of concentrated NaCl solutions from a membrane at the pH corresponding to the membrane isoelectric point.

4.2.2 Relevant Theory

The prediction of membrane performance has been an active area of research over the last two decades. During that time, the emphasis has shifted from empirical black box models based on irreversible thermodynamics (Levenstein et al., 1996) to models based on the extended Nernst-Planck equation (Tsuru et al., 1991) due to the ability of the latter to provide information related to properties of both the membrane and the process stream. The main purpose of such models is to incorporate as much physical realism of the membrane process as possible in order to better match measurable quantities to adjustable model parameters. One should always be mindful that the major limitation of nanofiltration modelling is the requirement for characteristic model parameters, such as pore radius and membrane charge, that are not readily measured at the near atomic length scales encountered. Similarly, the development of rigorous physical descriptions (such as Molecular Dynamics simulations) has been limited by the lack of detailed knowledge of the physical structure and electrical properties of real nanofiltration membranes and process streams. As a direct consequence, developments in modelling have moved in parallel with improvements in the measurement techniques employed for the characterisation of nanofiltration membranes and process streams, as only then will a check of the appropriateness of model parameters be possible.

The transport of ions is typically described using the extended Nernst-Planck equation:

$$j_i = -\frac{c_i K_{i,d} D_{i,\infty}}{RT}\frac{d\mu}{dx} + K_{i,c} c_i V \qquad [4.2.1]$$

where j_i is the ionic flux, c is the concentration, V is the solvent velocity and $K_{i,c}$ and $K_{i,d}$ are hindrance factors to account for the convection and diffusion inside a confined space. The electrochemical potential is written as

$$\mu_i = RT \ln a_i + V_{si} P + z_i F \psi + \text{constant} \qquad [4.2.2]$$

where R is the universal gas constant, T is the absolute temperature, V_{si} is the specific volume of the ion, P is the operating pressure, z is the ion valence, F is the Faraday constant and ψ is the electrical potential. Differentiation of Eq. [4.2.2] results in

$$\frac{d\mu}{dx} = \frac{RT}{c_i}\frac{dc_i}{dx} + \frac{RT}{\gamma_i}\frac{d\gamma_i}{dx} + V_{si}\frac{dP}{dx} + z_i F \frac{d\psi}{dx} \qquad [4.2.3]$$

Substitution of Eq. [4.2.3] into Eq. [4.2.1] yields

$$j_i = -D_{i,p}\frac{dc_i}{dx} - \frac{c_i D_{i,p}}{\gamma_i}\frac{d\gamma_i}{dx} - \frac{c_i D_{i,p}}{RT} V_{si}\frac{dP}{dx} - \frac{c_i D_{i,p}}{RT} z_i F \frac{d\psi}{dx} + K_{i,c} c_i V \qquad [4.2.4]$$

Eq. [4.2.4] represents the full extended Nernst-Planck equation and governs the transport of an ion in solution. In this case the equation has been specifically developed to describe the transport of an ion through a membrane pore, typical of that found in nanofiltration and loose reverse osmosis membranes. In order to solve the transport equation, the solute concentration at the feed side and permeate side, $c_i(0)$ and $c_i(\Delta x)$, of the membrane must be known. The entrance and exit of an ion from such a pore is an equilibrium process and this relationship is expressed as

$$\frac{\gamma_i c_i}{\gamma_i^o C_i} = \Phi_i \exp\left(-\frac{z_i F}{RT}\Delta\psi_D\right)\exp\left(-\frac{\Delta W_i}{k_B T}\right) \qquad [4.2.5]$$

The first two terms on the right hand side of Eq. [4.2.5] are the classic expressions for both steric and Donnan effects respectively (Deen *et al.*, (1980); Giddings *et al.* (1968); Donnan (1911)) and are generally well accepted throughout the literature [Oatley et al., 2012a]. The third term on the right hand side of Eq. [4.2.5] represents partitioning as a result of dielectric exclusion. There has been much debate over the nature of this effect and this has been summarised in a recent review [Oatley et al., 2012b] and will be further discussed later in this section.

The typical method for solving the equilibrium equation (Eq. [4.2.5]) is to assume ideal conditions and that electroneutrality exists in the bulk solution and inside the membrane pore, i.e.

$$\sum_{i=1}^{n} z_i C_i = 0 \text{ and } \sum_{i=1}^{n} z_i c_i = -X_d \qquad [4.2.6]$$

where X_d is the effective membrane charge density. When the assumptions are met, one may equate the Donnan potential for each ionic species and rearrange Eq. [4.2.5] in terms of a particular ionic species, in this case species 1:

$$c_{i(0)} = C_{i,w}\Phi_i'\left(\frac{c_{1(0)}}{C_{1,w}\Phi_1'}\right)^{\frac{z_i}{z_1}} \qquad [4.2.7]$$

and then substitute the result into Eq. [4.2.6]:

$$z_1 c_{1(0)} + z_2 C_{2,w}\Phi_2'\left(\frac{c_{1(0)}}{C_{1,w}\Phi_1'}\right)^{\frac{z_2}{z_1}} + \ldots + z_n C_{n,w}\Phi_n'\left(\frac{c_{1(0)}}{C_{1,w}\Phi_1'}\right)^{\frac{z_n}{z_1}} + X_d = 0 \qquad [4.2.8]$$

where $\Phi_i' = \Phi_i \exp\left(-\frac{\Delta W_i}{k_B T}\right)$

Thus, for a known feed solution, the pore entrance concentrations can be calculated from the solution of Eq. [4.2.8] and substitution to Eq. [4.2.7] for all ions. The same situation exists at the exit of the pore. The typical method for solution of the transport equation is to assume ideal conditions and neglect the effect of

pressure on the chemical potential. When this is the case Eq. [4.2.4] can be simplified and manipulated to give:

$$\frac{dc_i}{dx} = \frac{V}{D_{i,p}}\left[K_{i,c}c_i - C_{i,p}\right] - z_i c_i \left[\frac{\sum_{i=1}^{n} \frac{z_i V}{D_{i,p}}\left[K_{i,c}c_i - C_{i,p}\right]}{\sum_{i=1}^{n} z_i^2 c_i}\right] \qquad [4.2.9]$$

The solution procedure is then to numerically solve Eq. [4.2.9] for each ionic species and iterate the solution based on estimations of the permeate concentrations using Eq. [4.2.8] at the pore exit as a check mechanism. In the case of a neutral species, Eq. [4.2.9] simplifies to form an algebraic result:

$$R = 1 - \frac{C_{i,p}}{C_{i,w}} = 1 - \frac{K_{i,c}\Phi_i}{1 - \left[1 - K_{i,c}\Phi_i\right]\exp\left(-Pe\right)} \qquad [4.2.10]$$

where $Pe = \frac{K_{i,c}}{K_{i,d}} \frac{r_p^2}{8\eta D_{i,\infty}} \Delta P_e$ is a modified Peclet number, ΔP_e is the effective pressure driving force ($\Delta P - \Delta\Pi$), η is the pore viscosity and r_p is the pore radius.

4.2.3 Materials & Methods

All rejection experiments were carried out at laboratory scale using a frontal filtration set-up illustrated in Figure 3.3. In this experiment only the Membranology (Membranology Ltd., UK) cell was used, with the properties as described in Chapter 3. All membrane experiments were carried out at 25 ± 0.1 °C using a stirrer speed of 300 rpm. The membrane used for this study was the Desal DK (GE Osmonics, USA) nanofiltration membrane. NaCl was the sole solute used for the isoelectric study.

4.2.4 Results and discussion

Confirmation of the membrane isoelectric point

Pore dielectric effects and effective membrane charge density normally exhibit coupled behaviour. Thus, in order to evaluate a single effect their relationship must be decoupled. The membrane isoelectric point provides an opportunity to study

only dielectric effects due to the membrane charge density being effectively neutralised. The results of the streaming potential measurements are shown in Figure 4.2.1.

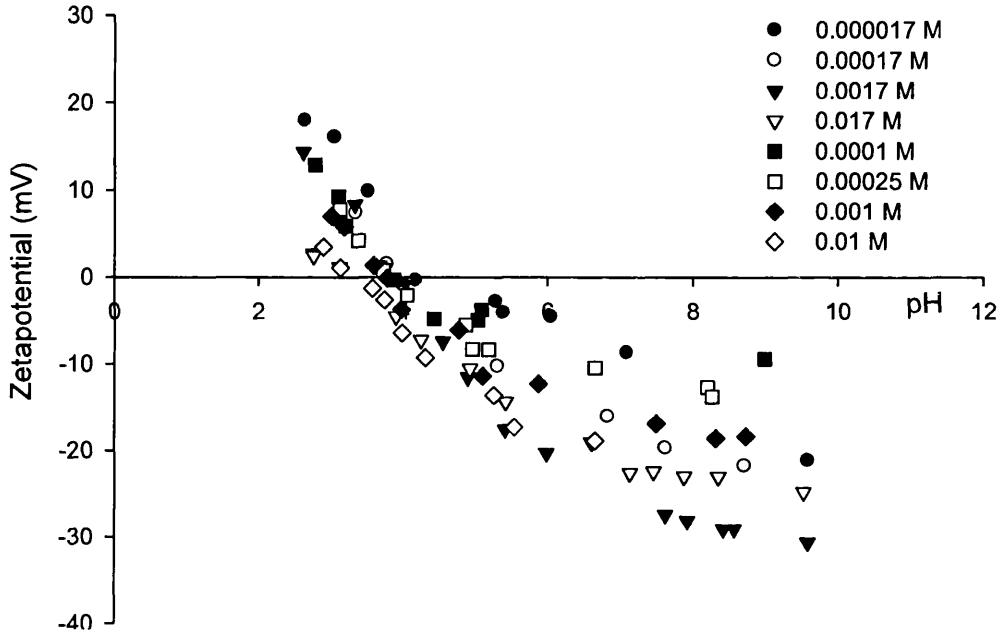

Figure 4.2.1: ζ-potential measurements at various concentrations and pH

The profile of ζ-potential for the salt studied exhibits positive values at low pH, becoming negative as pH rises and exhibits a negative plateau at high pH. The magnitude of the ζ-potential is proportional to concentration at high pH, with higher ζ-potential values observed at lower salt concentrations. The isoelectric point for each of the salt concentrations studied was in the range from pH 3.0 to 4.2, which is consistent with previous studies [Hagmeyer & Gimbel, 1999; Oatley et al. 2012; Bowen & Welfoot, 2002]. Interestingly, the isoelectric point appears to become lower as the concentration of salt increases. This suggests that a small quantity of the chloride ions in solution are adsorbing to the membrane surface. The ζ-potential versus chloride ion concentration in solution is illustrated in Figure 4.2.2 at pH 4.0 (data obtained by interpolation from experimental results).

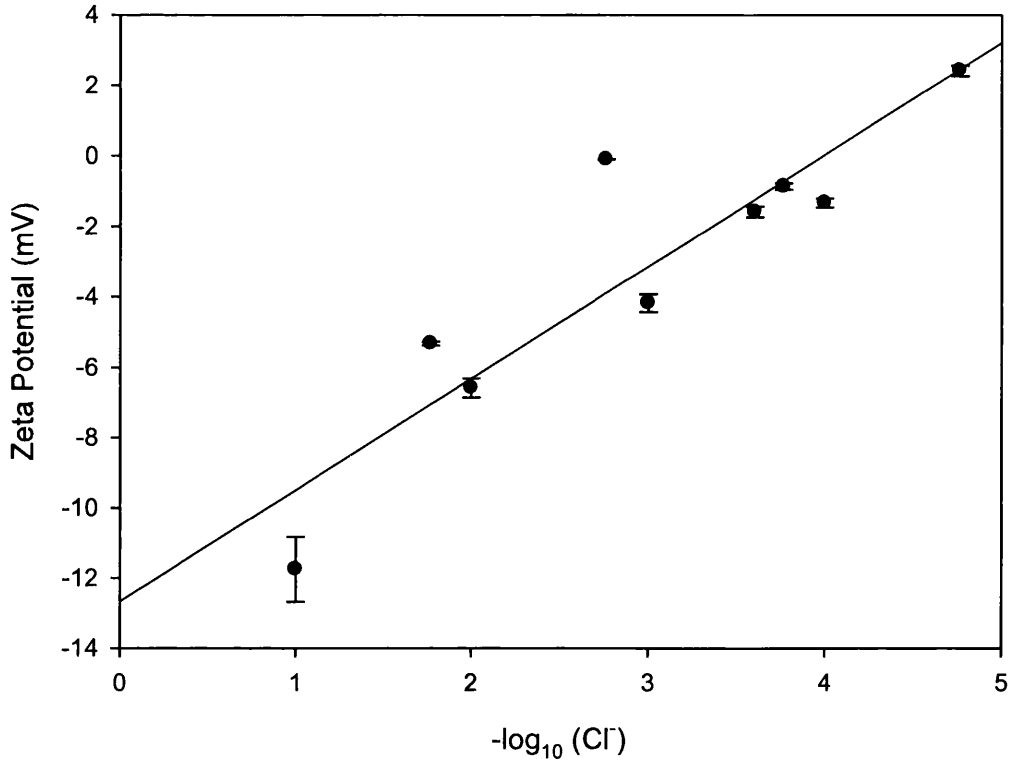

Figure 4.2.2: ζ-potential versus chloride ion concentration at pH 4.0

This information indicates that the ζ-potential at this pH is proportional to chloride ion concentration and, when taking experimental error into consideration, is linear in nature. This trend illustrates that chloride ion adsorption is taking place and that the shift in the isoelectric point is real and not simply a function of experimental error.

The rejection data vs pH for constant salt concentration at fixed pressure is shown in Figure 4.2.3. The rejection data for the two concentrations studied exhibits a sharp fall in magnitude in the region of pH 3.8 to 4.1. This fall in rejection is attributed to the neutralisation of the membrane at the isoelectric point facilitating free passage of the salt through the membrane by diminishing the effects of charge repulsion and reducing Donnan potential. The evaluated isoelectric point using this method is in agreement with that obtained from the streaming potential method described previously and subsequent experimentation was conducted in the pH

range 3.9 to 4.0 which is representative of the isoelectric point where the membrane charge will be at or very near to neutrality. Thus, negating the effects of the membrane charge density on rejection.

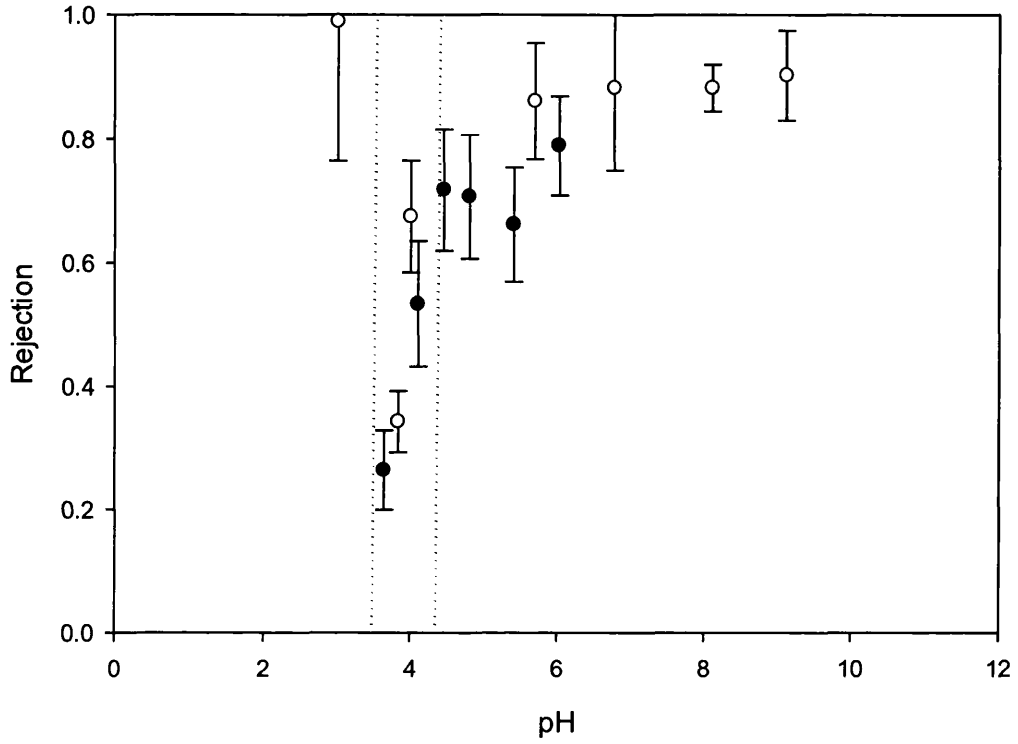

Figure 4.2.3: NaCl rejection at 10 bar (o 0.001 M; • 0.6 M)

Electrolyte rejection and flux at the membrane isoelectric point

The rejection of various concentrations of salt at the membrane isoelectric point is shown in Figure 4.2.4. Figure 4.2.4 represents the rejection data plotted as a function of applied pressure and clearly indicates that rejection increases as a function of applied pressure and then attains a plateau at high pressure as expected. More interestingly in this case is the fact that the salt rejection actually decreases significantly as the concentration is increased. This behaviour is unexpected and the significant fall in rejection indicates that this is not the result of any amount of experimental error and is a true observation. Figure 4.2.5 illustrates the same experimental data but this time plotted as rejection vs. concentration at

different pressures. From this plot, one can clearly observe that the rejection falls as concentration increases. The plot also indicates that there is a pressure dependence to the behaviour in that at high pressures the fall in rejection is linear and at low pressure the fall in rejection is more exponential in nature.

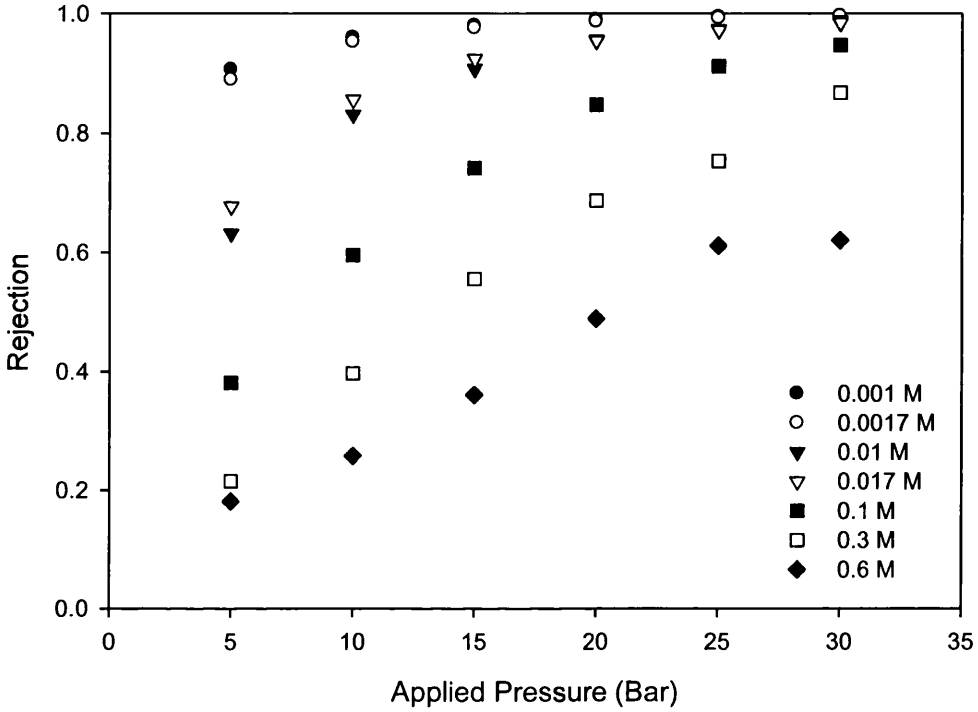

Figure 4.2.4: Real rejection versus applied pressure for various solutions of NaCl at the membrane isoelectric point

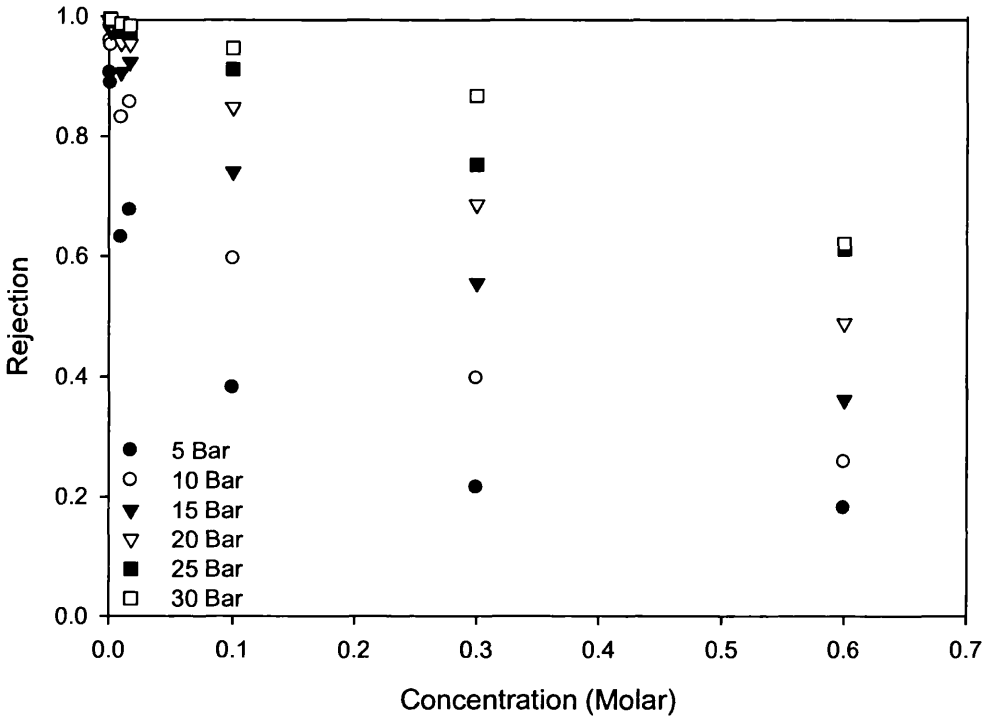

Figure 4.2.5: Real rejection versus concentration for various solutions of NaCl at the membrane isoelectric point

The observed trends demonstrate that there is a phenomena occurring that is not accounted for in the model described in Section 4.2.4. The fact that the rejection is falling with increased concentration would be very typical for a charged membrane and this phenomena is well documented [Bowen and Welfoot, 2002; Bowen et al., 2004; Cheng et al., 2012]. However, as Eq. [4.2.11] would suggest, the rejection for a neutral system should be independent of concentration. So the obvious question to pose is why is this behaviour occurring? One explanation for the behaviour may come from the fact that fixed charge on the membrane is finite in nature, i.e. the charge itself is at a fixed location. Normal assumptions when modelling charge is that the charge density is the same in all places and the charge is considered to be a continuum phase. However, in reality the charge is developed from the dissociation of acidic and basic groups as well as from point charges of adsorbed ions. Thus, at the isoelectric point where the global fixed charge is minimal. There may be point charges that can be screened by the increase in concentration and that is affecting

rejection by allowing the transport of ions. The simplest explanation to consider is that the description of dielectric exclusion is incorrect. The Born model used to describe dielectric exclusion in Eq. [3.1] is represented as

$$\Delta W_i = \frac{z_i^2 e^2}{8\pi\varepsilon_o a_i}\left(\frac{1}{\varepsilon_p} - \frac{1}{\varepsilon_b}\right) \qquad [4.2.11]$$

where ΔW_i is the ion solvation energy barrier, z is the ion valence, a_i is the solute hydrodynamic radius (Stokes Radius), e is the elemental electron charge, ε_o is the permittivity of free space, ε_p is the pore dielectric constant and ε_b is the bulk dielectric constant. Simple inspection of Eq. [4.2.11] indicates that there is no concentration term present. Indeed, on review of the assumptions contained in the original Born model [Born, 1920] then the model considers the transport of a single ion from a vacuum into a dielectric media, i.e. there is no implicit account for the fact that multiple ions exist in the system considered here. This being the case, there could well be a justified argument to revisit the original assumptions of the Born model and introduce multiple ions to the derivation.

Other descriptions for dielectric exclusion exist, most notably those considering the phenomenon of image forces. The descriptions of dielectric exclusion as a result of image forces are quite complex in nature and the most simple form is that for slit like pores [Yaroshchuck, 2000; Vezzani and Bandini 2002].

$$\Delta W = W(0^+) - W(0^-) = r_B\left\{\kappa(0^-) - \frac{\varepsilon_b}{\varepsilon_p}\left[\kappa(0^+) + \frac{1}{r_p}\ln\left(1-\gamma e^{-2r_p\kappa(0^+)}\right)\right]\right\} \qquad [4.2.12]$$

where r_B is the Bjerrum radius, κ is the inverse Debye length, (0^-) and (0^+) represent locations just outside the membrane and just inside the membrane respectively, with

$$r_B = \frac{F^2}{8\pi\varepsilon_b RTN_A},\ \gamma = \frac{1-\varepsilon_m/\varepsilon_p}{1+\varepsilon_m/\varepsilon_p},\ \kappa(0^-) = F\sqrt{\frac{2I(0^-)}{\varepsilon_b RT}},\ \kappa(0^+) = F\sqrt{\frac{2I(0^+)}{\varepsilon_b RT}} \qquad [4.2.13]$$

where I is the ionic strength. Eq. [4.2.12] represents two different mechanisms of interaction. The first two terms in the brace parenthesis represent the difference between the reciprocal of the Debye lengths, calculated inside the nanopore and in the bulk solution. The parameter κ is derived from the Debye-Huckel theory and is related to the activity coefficient for a single charged ion and describes the changing ion-ion interactions between the bulk and pore solutions. The third term describes the ion-polarisation energy of interaction [Dukhin et al., 1988]. The exponential term describes the typical decay of an any screened electrical field; $2r_p\kappa(0^+)$ is the decay rate [Israelachvili, 1991] and is the ratio between pore size and Debye length inside the nanopore. The screening is most relevant when the dimensionless parameter approaches unity, i.e. for large pores or in this case concentrated solutions. Thus, this equation describes a screening effect on the dielectric partitioning that can explain the behaviour observed in Fig 4.2.5. Although the existence of image forces is questionable for NF membranes (Bowen and Welfoot, 2002), this work suggests that the Yaroshchuck model (Eq. [4.4.12]) may be the better current description of dielectric exclusion from a fundamental basis.

4.2.5 Conclusions

Accurate models of nanofiltration are required for the *ab initio* design, optimisation and scale up of effective industrial processes. The most widely accepted models of nanofiltration are based upon the extended Nernst-Planck equation and have been shown to be highly effective for the modelling of simple salt solutions in the laboratory and have yet to be fully demonstrated for separations of industrial importance.

Dielectric exclusion of a nanofiltration membrane has been investigated at the membrane isoelectric point. This effectively neutralises the membrane charge and decouples the effects of membrane charge and dielectric exclusion, allowing a systematic independent study of dielectric exclusion only. A rejection study for a range of salt concentrations was made using sodium chloride. This study revealed that a significant drop in rejection was observed with increasing concentration. This highlighted the fact that there may well be some screening of the dielectric behaviour at higher concentrations that is not properly accounted for using the

Born model. Other models of dielectric exclusion based on image forces were shown to be capable of representing this phenomenon and may be more appropriate for use in modelling from a fundamental stand point.

4.3 Overall Conclusions and Recommendations

The theoretical descriptions used to describe hydrodynamic forces in nanofiltration have been shown to be feasible through the experimental study undertaken in this section. The work in this case focused on independently identifying the size of the solute and pore size prior to performing a series of rejection experiments using single solute PEG solutions of differing molecular weights. The work may be further investigated by using a range of different solutes (sugars, etc.) and pore sizes (different membranes) in order to verify the that the relationship is valid across the whole nanofiltration range.

Furthermore the investigation of the nanofiltration dielectric exclusion at the membrane isoelectric point showed a significant decrease in ion rejection with increasing concentration. This phenomenon suggested a screening of the dielectric behaviour at higher concentrations, a finding that is not accounted for using the Born model. The screening effect observed suggested that other models of dielectric exclusion based on image forces may be more appropriate for use from a fundamental stand point.

5.0 Feasibility of membrane technologies as a technique for rapid isolation and purification applied to sustainable drug discovery from natural products

The work presented in the previous chapters of this thesis have mainly focused on the theoretical aspects of membrane technology, paying particular attention to nanofiltration. The work presented in the following chapters attempts to apply nanofiltration for the separation and purification of bioactive compounds from a variety of natural sources. The first application attempts to separate an extracellular bioactive compounds from a fungal fermentation. The nanofiltration technology is to be directly compared to the traditional separation method of solvent extraction through the application of the products of each method as a pest control agent. Reported use of the technology for such applications is limited and as such the feasibility of membrane technology for such a process is studied here. Nanofiltration, due to the separation range, is a perfect candidate for the separation of small (<1000 MW) bioactives, however, the complex nature and high fouling potential of biological feeds undoubtedly provides a significant challenge to this technology. The selection of the appropriate membrane for this study is based on predicted theoretical rejections derived from the membrane and solute physical properties. The work contained within these experimental chapters applies the theoretical aspects of nanofiltration to real separations of scientific interest which allows the separation performance of the technology to be evaluated.

5.1 Introduction

Natural products are typically classified as organic materials derived from animal or plant extracts. The therapeutics derived from natural products are generally classified as macromolecules or small molecules. Macromolecules are large complex chemical structures derived from fermentation or extracted directly from animal or plant tissues. These consist of, but are not limited to, hormones, enzymes, monoclonal antibodies and small interfering RNAs (siRNAs). On the other hand, small molecules are typically primary or secondary metabolites produced either by fermentation or chemical synthesis. Examples of these molecules are

antibiotics, antifungals, antivirals, antidepressants, steroids, inhibitors, antibodies and antagonists etc. and are generally classified in the size range less than 1000 Da. Most of the leads from natural products that are currently in development have come from either plant or microbial sources (Harvey, 2008). One particular microbial source that has been highly productive for the discovery of new therapeutics are the fungi; these have been a valuable source of active compounds and are best known for the beta-lactam group of antibiotics, most notably penicillin. A plethora of other materials derived from higher fungi have been used for the treatment of hepatitis, hypertension, hypercholesterolemia, gastric cancer, HIV, hepatic and renal conditions, respiratory and pulmonary disease and some immune system disorders. The diversity of products and treatment areas currently known from fungi has been reviewed by Aly *et al.* (2011). Currently the belief is that only 5% of fungi are classed as producers, with the rest waiting to be tapped for their benefit (Hawksworth, 2001). These have been a valuable source of molecules of clinical interest some of which have been developed into medicines such as the immune modulating drugs cyclosporin and myriocin.

Metarhizium is one of the most common hypocrealean entomopathogenic fungi found worldwide and a source of a number of compounds of significant interest. For about 130 years, entomopathogenic fungi and especially *Metarhizium anisopliae*, have been used for biocontrol of pest insects (Zimmerman, 2007; Faria and Wraight, 2007), however, only recently the full potential and many advantages of using a natural product as a pesticide has seen commercial scale application. There are currently more than 47 *Metarhizium* based products available for pest management (Faria and Wraight, 2007). Pests are traditionally controlled by chemical pesticides, however, the widespread use of often synthetic pesticides have resulted in growing insecticidal resistance, environmental contamination, damage to non-target species and risks to human health through contamination of food and water sources (Lubeck et al., 2008). These factors combined with the relative availability and cost of synthetic pesticides in the developing world has led to the development of biological control agents. The issue of pest control is very serious despite only a small number of athropods being classed as pest species.

Despite the small numbers, pests still cause major devastation destroying up to 18% of the world annual crop production, contributing to nearly 20% loss of stored food grains, and causing approximately US$ 100 billion of damage each year (Schrank and Vainstein, 2010). Further to arthropod pests, disease vectors such as mosquitoes, ticks and fleas are of major concern, particularly in the developing world.

Metarhizium anisopliae is known to produce a wide variety of secondary metabolites including swainsonine (a potent and highly specific alpha-mannosidases inhibitor with anti-metastatic characteristics, potential in the treatment of cancer and AIDS (Tamerler-Yildir et al., 1997), destruxins which incidentally were first identified from *M.Anisopliae* (potential to treat cancer, osteoporosis, Alzheimer's disease, and hepatitis B (Wang et al. 2012)) and iminosugars (known glycosidase inhibitors and diabetic control agents, potential for immune modulation, antiviral and cystic fibrosis applications (Nash et al., 2011)). The structures of some of the metabolites of *Metarhizium* are shown below in Figure 5.1. The metabolites shown in Figure 5.1 display a neutral charge under neutral and alkaline conditions, however would display a slight positive charge under acidic conditions due to the effect of coordinate bonding between protons and the lone pair of the nitrogen. *Metarhizium's* metabolite profiles depend on the species, strain, cultural conditions and isolation protocols. Several workers (Jackson, 1997; Tamerler and Keshavarz, 1999; Singh and Kaur, 2011; Jackson and Jaronski, 2012) have tried to optimise production yields of the compounds of interest however; in most cases the amounts produced are relatively small. The fact that such low amounts are secreted contributes to the frustration for those involved with risk assessment studies due to the expense of producing standards and many compounds being unavailable commercially. Even those standards that are available are extremely expensive.

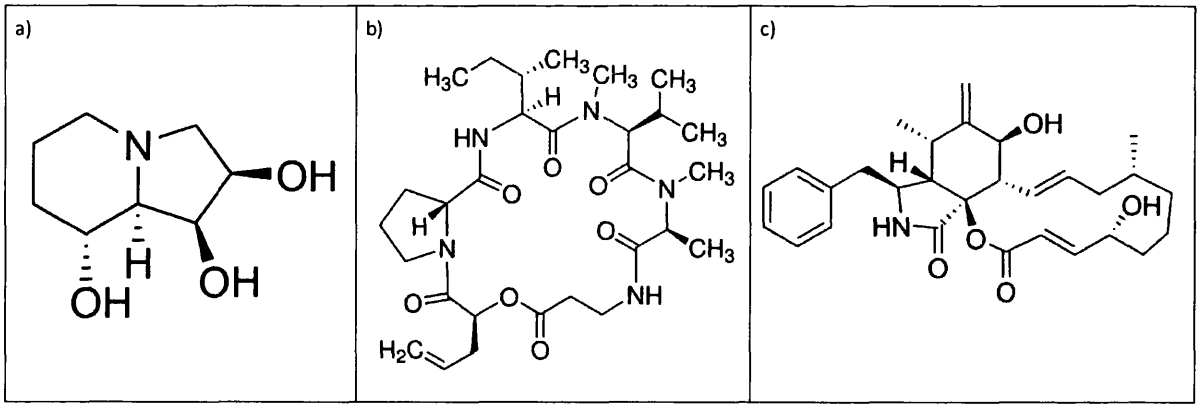

Figure 5.1: Structures of a) Swainsonine (MW - 173), b) Destruxin A (MW - 577) and c) Cytochalasin B (MW - 479)

These secondary metabolites have long been suspected to act like virulence factors in entomopathogenesis and to function as toxins which kill the host species and hence act as a potent bio-control agent (Rohlfs and Churchill, 2011). For example, destruxins produced by *M. Anisopliae* have been reported to cause paralysis and subsequent death at sub-nanomolar concentrations, furthermore destruxins have exhibited anti-feedant activity therefore hasten death by starvation (Molnar et al., 2010). However, not all fungal secondary metabolites produce such significant mortality effect, although should not be discounted in the process of mycosis. *Metarhzium* metabolites have been shown to suppress the insect immune system and therefore may enhance the mortality effect of some secondary metabolites (Pal et al., 2007). *Metarhizium anisopliae* is naturally found in soil and therefore predominantly infects soil-dwelling insects, however, recent years has seen increased research into the artificial application of fungi as a bio-control agent (Glare et al., 2010; Fernandes et al., 2012), in both laboratory (Andres et al., 2014) and field studies (Erler et al., 2013; Camargo et al., 2014). The majority of studies focus on the infection of insects using fungal conidia (cellular matter) as opposed to the external secondary metabolites produced by the fungus.

Interestingly, Schrank and Vainstein (2010) identified that "the reasons in the delay to adopt biocontrol strategies are laborious work of isolating and identifying potential biocontrol agents and the developing of suitable formulations to maximize the efficiency of the bioproducts." However, the authors did identify that bio-control using entomopathogenic fungi is now a reality. The long development

times may be partially attributed to the separation, purification and detection methods used to elucidate these compounds of bioactive interest (Duarte et al., 2012). Traditionally the first stage in the traditional bioactive discovery process from a natural product source is to develop lead compounds. Simplistically, this is the production of a molecule (or series of molecules) followed by an assay test for activity against the cell, organism, biological process or disease area of interest. The activity test does not require ultra-high purity and will demonstrate activity from a relatively crude mixture of compounds. Clearly, once activity has been determined, ultra-high purity is required to identify the molecule generating activity. A typical production scheme for molecules to feed into this process is highlighted by Chen *et al.* (1999) and comprises of fermentation, blending and centrifugation, solvent extraction, ion-exchange chromatography, silica gel chromatography and semi-preparative HPLC to produces a series of molecule fractions. This process as outlined is costly, labour intensive, solvent intensive, has low mass efficiency and suffers the drawback of solvent bias in terms of molecule polarity, i.e. polar molecules of potential interest will remain in the aqueous phase following extraction and will be lost to waste (Duarte et al., 2012; Murthy & Manohar, 2014). Furthermore, there is growing interest in reducing the quantity of organic solvent used due to rising solvent purchasing and disposal costs coupled with increased regulatory restrictions (Khajeh, 2011). Membrane processes have the potential to recover the small molecules of interest from the fermentation broth without the need for solvent extraction or chromatographic processes, thus delivering the same product as the traditional process but with reduced processing time, reduced labour and reduced solvent inventory leading to less energy consumption and higher mass efficiency leading to ease of scale up. Furthermore, membrane technology offers a particular environmental benefit when compared with solvent extraction in terms of scale up. The question arises, why has membrane technology not been used so little for bioactive discovery before now'? The simple explanation for this is that membranes are a relatively young technology and the scientists working in the field of drug discovery will either have had little exposure to them or are reluctant to change experimental methods. Similarly, membranologists and engineers are unlikely to be working in the bioactive discovery area. Thus, this work

illustrates the significant benefits that may occur when cross disciplinary research is undertaken.

As seen previously, the nominal molecular weight cut-off of an NF membrane is in the range 100-1000 Da, indicating that the NF membrane active layer has an approximate pore size of 1 nm and therefore presenting an ideal separation method for small bioactive compounds. NF membranes have been extensively exposed to chemical pesticides, however, only for their removal from waste waters (Plakas and Karabelas, 2012). The relative infancy of NF has seen little application of the technology for the separation and purification of bioactive compounds. An excellent example of the use of NF as a replacement to a traditional solvent extraction process was reported by Tsibranska et al., (2011) and Tylkowski et al., (2011) where the membrane method produced a final product with concentrations 3-4 times higher than the traditional extraction process, whilst still maintaining the antioxidant properties. Tylkowski et al. (2010) not only highlighted the potential of nanofiltration for the purification of biaoctive compounds but also the potential of the technology in helping identify and investigate the properties of individual compounds of low concentration that may otherwise be lost through traditional extraction methods. The range of bioactive metabolites produced by fungi suggest nanofiltration is a separation process with true potential, as many of the metabolites produced have a MW below 1000 (Figure 5.2).

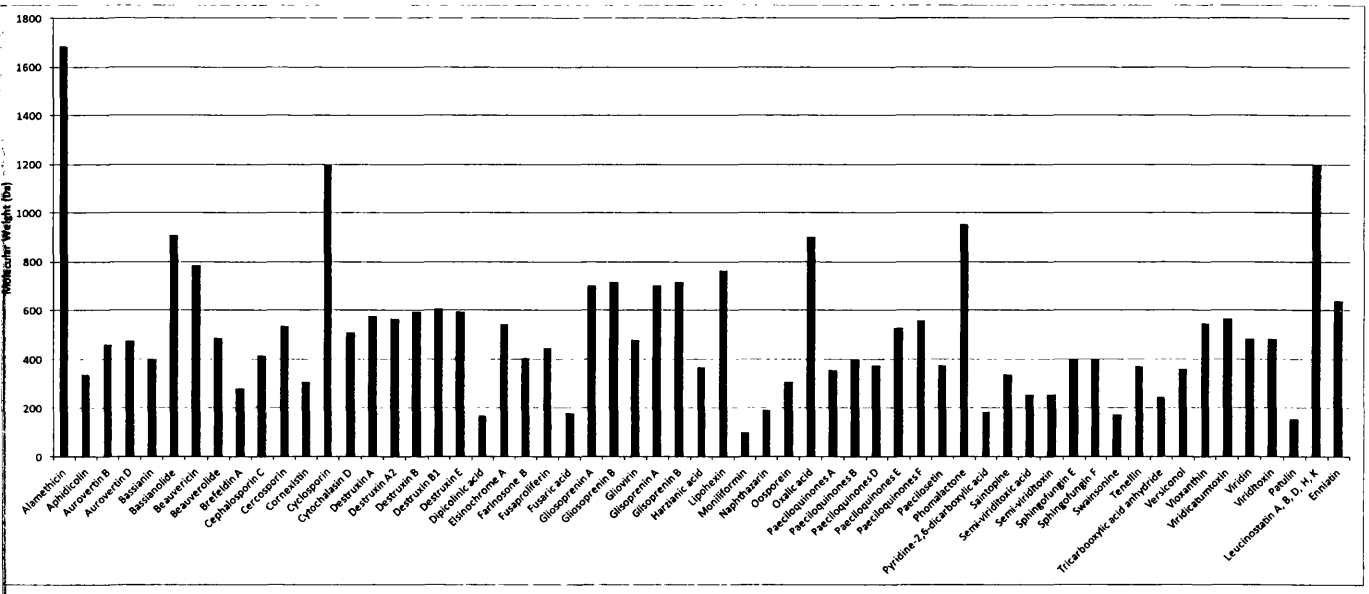

Figure 5.2: Molecular weights of various metabolites produced by fungi (Personal correspondence from Prof. T.M. Butt, Unpublished)

The aim of this study is to assess the feasibility of introducing NF membrane technology for the purification of fungal small molecules and subsequent product bioactivity in a biological assay as a pest control agent. Ideally, the membrane process should be capable of isolation and concentration the compounds of interest in the size range less than 1000Da. A comparison will be made to a traditional recovery process to assess the impact and potential sustainability benefits of the membrane process as well as the performance of the extracts in bioassays.

5.2 Materials and Methods

5.2.1 Fungal material

Three strains of *Metarhizium* were evaluated: *M. brunneum* strain BIPESCO 5 (= V275), *Metarhizium anisopliae* strains ARSEF 4556 and ARSEF 23. The fungi were cultured on SDA and incubated at 25 ± 1°C under darkness condition. For all experiments used conidia had over 95% viability.

The three strains above were produced in three different media which differed in their composition and carbon:nitrogen ratio. The media were: Czapek dox+peptone, Czapek dox+yeast, and 10:1 (C:N ratio) media. The 250ml media was

inoculated with conidia to give a final concentration of 10^7conidia/ml. The 500 L flasks were incubated in a rotary shaker at 110 rpm for 7 days at 25ºC. The cultures were filtered through two layers of Miracloth (Millipore, Bedford, MA) and then through Whatman's No. 1 filter paper (Whatman, Newton, MA). The crude extracts were filtered through a 0.2 µm Millipore filter and stored at -20°C.

Conidial suspensions were prepared by scraping conidia from stock cultures with a sterile spatula into 0.03% aqueous Tween 80 (Sigma-Aldrich, Dorset, UK) then vortexed for 5 minutes to ensure homogenous suspension. The spore concentration was determined using a haemocytometer (Neubauer bright-line, Superior Marienfeld, Lauda-Konigshofen, Germany) and adjusted to $1x10^8$ spores mL^{-1} with additional Tween 80. Each flask was then inoculated with the prepared $1x10^8$ spores mL^{-1} conidial suspension. The flasks were incubated in a rotary shaker (Innova 44, New Brunswick Scientific, CT, USA) at 28 ± 2 °C and 130 rpm for 8 days. After this period, the fermentation broth was filtered under reduced pressure using a Büchner funnel and Whatman #1 filter. The dry weight of biomass was determined by pre-weighing the filter paper and then drying the resulting filter paper and residue in a vacuum oven for 24 hours. The remaining liquors, termed the culture filtrate, were then ready for onward processing by either the traditional solvent method or membrane processing and contain ~0.1%wt of products.

The traditional isolation process of bioactive metabolites

The solvents selected for the extraction process were ethyl acetate (EtOAc) and dichloromethane (DCM), both were HPLC grade and obtained from Fisher Scientific UK (Loughborough, UK). The culture filtrate was extracted in a separating funnel by addition of solvent (0.5:0.5 EtOAc:DCM) in a 1:1 ratio (volume basis) to the culture filtrate and then vigorously mixed for 5 minutes. The resultant mixture was allowed to settle for a minimum of 30 minutes and the aqueous layer was then removed and the solvent layer transferred into a rotary evaporator flask and capped. The aqueous layer was then returned to the separating funnel and the process was repeated. The resulting solvent mixture was then dried using a rotary evaporator

(Buchi Roatvapor R-200, Buchi UK Ltd, Oldham, UK). The dried extract was weighed and then subsequently re-dissolved in a small quantity of methanol (<5mL) and transferred to an Eppendorf vial prior to being dried overnight under vacuum in a desiccator. The resulting product vials were then stored at -18°C.

Table 5.1: Full range of strains and media used throughout the study

Sample	Name
1	**4556 + Yeast**
2	**4556 + Peptone**
3	**4556 + 10:01**
4	**Arsef 23 + Yeast**
5	**Arsef 23 + Peptone**
6	**Arsef 23 + 10:01**
7	**V275 + Yeast**
8	**V275 + Peptone**
9	**V275 + 10:01**

5.2.2 Other measurements

HPLC assay: The known metabolites were purified by HPLC using a modified method (Taibon et al., 2013). The chromatographic profiles of the samples were detected with a Agilent 1100 HPLC system, equipped with a C18 reverse phase column (Acclaim, silica, particle size: 5µm, pore diameter: 120 Å, length: 4.6 X 250 mm) and UVD diode array detector at a flow rate of 1 mL min^{-1}. The mobile phase was a linear gradient of pure (RO) water and acetonitrile (Fisher Scientific UK, Loughborough, UK) each containing 0.02 vol% acetic acid, and a gradient of (time) t=0 min 75%A, t=6.5 min 60%A, t=8 min 50% A, t= 8.5 min 25%A, t=10 min 5% A, t=30 min 2% A, maintained for 20 minutes, followed by a post run of 30 minutes of 75% A for column equilibrium prior to running next sample. 10 µl of each sample was injected and monitored at a wavelength of 215, 254 and 280 nm. Standards of Destruxin E, Destruxin A, Swainsonine, and Cytochalasin (Sigma-Aldrich, Dorset, UK)

were used to produce calibration curves for the known metabolites of the fungal strains.

Biological assay against mosquito larvae: Assay was performed following the World Health Organization method (WHO, 1996) by using 24-well plates (Nunc, Roskilde, Denmark) with 10 ml of water. Fractions obtained by solvent extraction and the membrane process (NTR-7450 membrane) of the *M. brunneum* strain ARSEF 4556 were assayed against 6 *Culex quinquefasciatius* larvae per medium. 5 mg of each freeze dried extract was diluted in 1 ml of methanol, and 100 µl of the mix were transferred to each well. Solvent control was assessed and the standards dtx A and E, swansonine and cytochalasin were evaluated at 100 ppm. The assay was performed in triplicate in darkness and larval mortality and morphological deformations were recorded daily for 96h.

5.2.3 Membrane selection and theoretical rejection

The selection of an appropriate nanofiltration membrane is key to the success of the separation. The general aim of this experimental work was to select one nanofiltration membrane that would allow the permeation of the bioactive compounds and one that would retain the bioactive compounds of interest, i.e. remove higher molecular weight species with the first membrane and then concentrate the molecules of interest with the second. Therefore, the NTR-7450 (MWCO - 1000 Da) membrane was selected in order to allow the permeation of the compounds of interest while the NF 200 (MWCO - 300 Da) was selected in order to retain these compounds and concentrate.

The theoretical rejection performance of these membranes was investigated using equation 4.1.27. In order to use the equation the bioactive compounds were treated as neutral species. The theoretical rejection of destruxin E and cytochalasin by the NTR-7450 and NF 200 membranes were investigated. The use of theoretical descriptions for the prediction of membrane performance must be considered as a guide as the theoretical models do not consider such phenomena as fouling and concentration polarisation. The NTR-7450 membrane manufacturer reports a MWCO of 1000, however upon further investigation (Appendix A2) the membrane

is seen to have a MWCO of 400 Da. This is a significant difference and as such has a dramatic effect on membrane performance. The manufacturers reported MWCO of 1000 Da gives a theoretical rejection of 85.5 and 94.5% for cytochalasin and destruxin E at 20 bar respectively. However, if the experimentally measured MWCO of 400 Da is used the theoretical rejections of cytochalasin and destruxin E both rise to 100% respectively. The theoretical rejections of cytochalasin and destruxin E are both 100% at 20 bar for the NF200 membrane. The theoretical rejection values obtained would suggest that the two membranes selected should both reject a significant quantity of the bioactives studied.

5.3 Results and Discussion

5.3.1 Initial scoping work

The most simplistic membrane process that could be employed for the recovery of molecules of interest from the culture filtrate is direct NF. The initial scoping work intended on implementing a process that allowed the transport of the compounds of interest into the nanofiltration permeate, hence the selection of the fairly loose nanofiltration membrane the NTR-7450 with a reported MWCO of 1000 (Bargeman et al., 2005). NF was applied directly to the culture filtrate solution and the resulting average membrane flux was found to be low at 0.3 LMH bar^{-1} (V275 + 10:01 media; NTR-7450 membrane). The membrane flux rate decreased quickly during processing reaching 0.2 LMH bar^{-1} after processing 80 mL of culture filtrate resulting in prohibitively slow process. Furthermore the membrane experienced significant visual fouling as shown in figure 5.3.

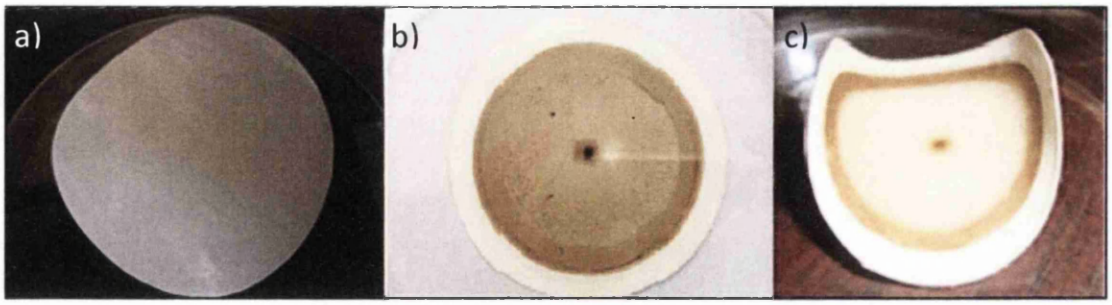

Figure 5.3: Condition of the NF membrane (NTR-7450): a) clean membrane prior to processing, b) fouled membrane following direct NF, c) fouled membrane following NF with pre-treatment.

Figure 5.3a shows the clean membrane prior to any filtration activity and illustrates that the membrane is pure white with no blemishes or fouling. Figure 5.3b shows the membrane following direct NF and the membrane is now heavily fouled with a brown scum layer coating the surface of the membrane believed to be residuals from the fermentation media or large molecular weight compounds. This heavy fouling layer is the principal cause of the reduction in membrane flux. In order to improve the NF process the fouling layer needs to be reduced or, if possible, removed altogether. The reduction in fouling layer displayed in Figure 5.3c suggests that the pre-treatment stage is removing large macromolecules from the NF feed. Therefore, the fouling observed in Figure 5.3b may be thought of as surface fouling. However, the slight discolouration shown in Figure 5.3c would suggest that there is some surface fouling still occurring. The effect of pore fouling may not be discounted, however this would need to be further investigated through the observation of flux decline during processing followed by flux recovery during cleaning. Surface fouling often exhibits a much more sudden flux decline than pore fouling which would allow the effect of fouling to be examined (Schäfer et al., 2004).

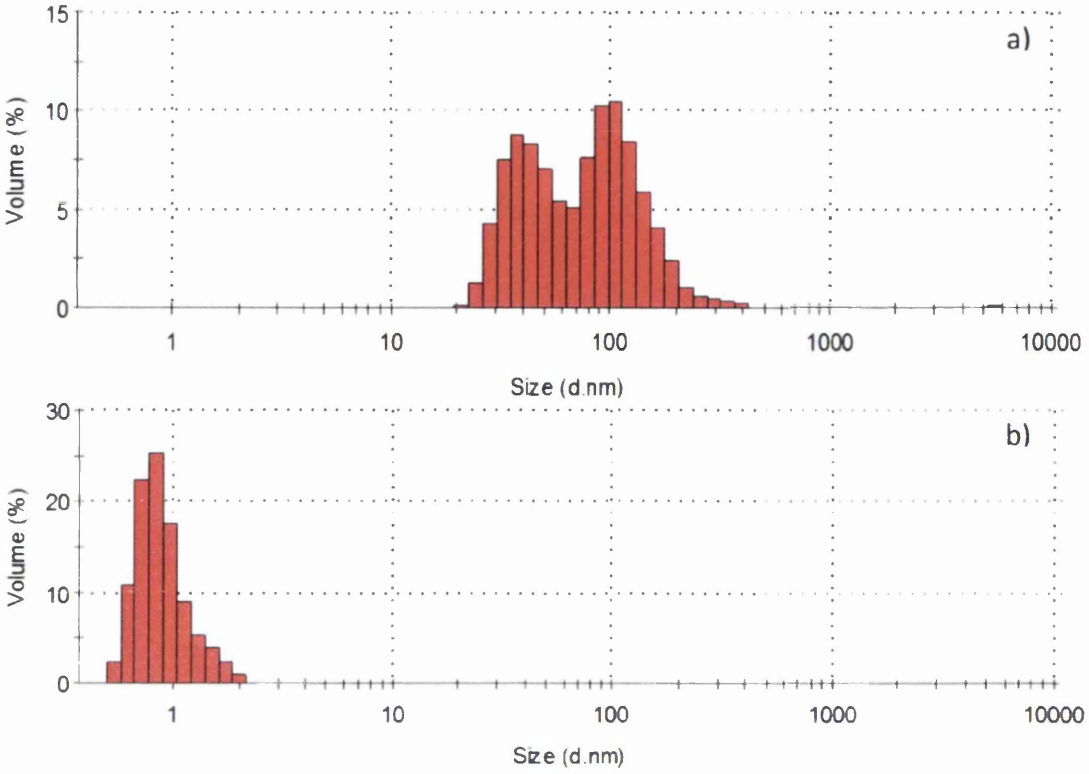

Figure 5.4: Dynamic light scattering measurements for a) the culture filtrate solution and b) permeate solution from the pre-filtration process.

Figure 5.4a shows the particle size analysis for the culture filtrate solution obtained by DLS measurement and clearly indicates a great deal of material in the size range 20 to 400 nm. This material is most likely unused raw materials, cellular debris and secreted compounds from the organism during the fermentation. In order to remove these erroneous materials a 4000 MWCO ultrafiltration process was introduced as a pre-treatment stage to the NF process. Figure 5.1.4b shows the results from a DLS measurement of the permeate solution from the pre-treatment process and illustrates that the materials in the 20 to 400 nm range have been removed and there is now only materials in the 0.5 to 2 nm range. Note that due to the nature of the DLS measurement, the larger material in the 20 to 400 nm range swamps the signal and thus the material in the 1 nm range is not visible until these larger materials are removed. The NF process was then implemented following pre-treatment and the fouling was significantly reduced, as indicated in Figure 5.3c. Some fouling is still observed in figure 5.3c; however this is inevitable in the

processing of a biological solution using frontal filtration where there is no tangential flow to help scour and remove any deposits. Clearly there is further work that can be done to optimise this process; however, the aim of this work is to demonstrate process feasibility only, so the residual quantity of fouling and membrane performance was deemed acceptable in order for the experimental work to continue.

Further to the inclusion of a prefiltration stage the initial scoping work suggested that not all of the compounds of interest were permeated through the NTR-7450 membrane and were being retained by the selected membrane. The prefiltration membrane was selected to account for the potential of retaining the compounds of interest by selecting a membrane that ensures maximum permeation of the compounds while rejecting all compounds below a MW of 4000, essentially attempting to produce a fairly pure fraction. To further investigate the potential of the compounds being retained a tight NF membrane (NF 200) was selected to try and obtain the highest rejections possible of the compounds.

5.3.2 Development of the NF process

Figure 5.5 shows the process flow diagram for both the traditional (Figure 5.5a) and the membrane (Figure 5.5b) process. In both the traditional and membrane process an initial volume of 200 mL enters the process. The solvent extraction process however, used two volumes of organic solvent per volume of fermentation media, therefore requires the addition of 400 mL of solvent. This results in a waste of 400 mL that is not associated with the membrane process. The membrane process requires the use of no additional solvents due to the separation being size-based alone.

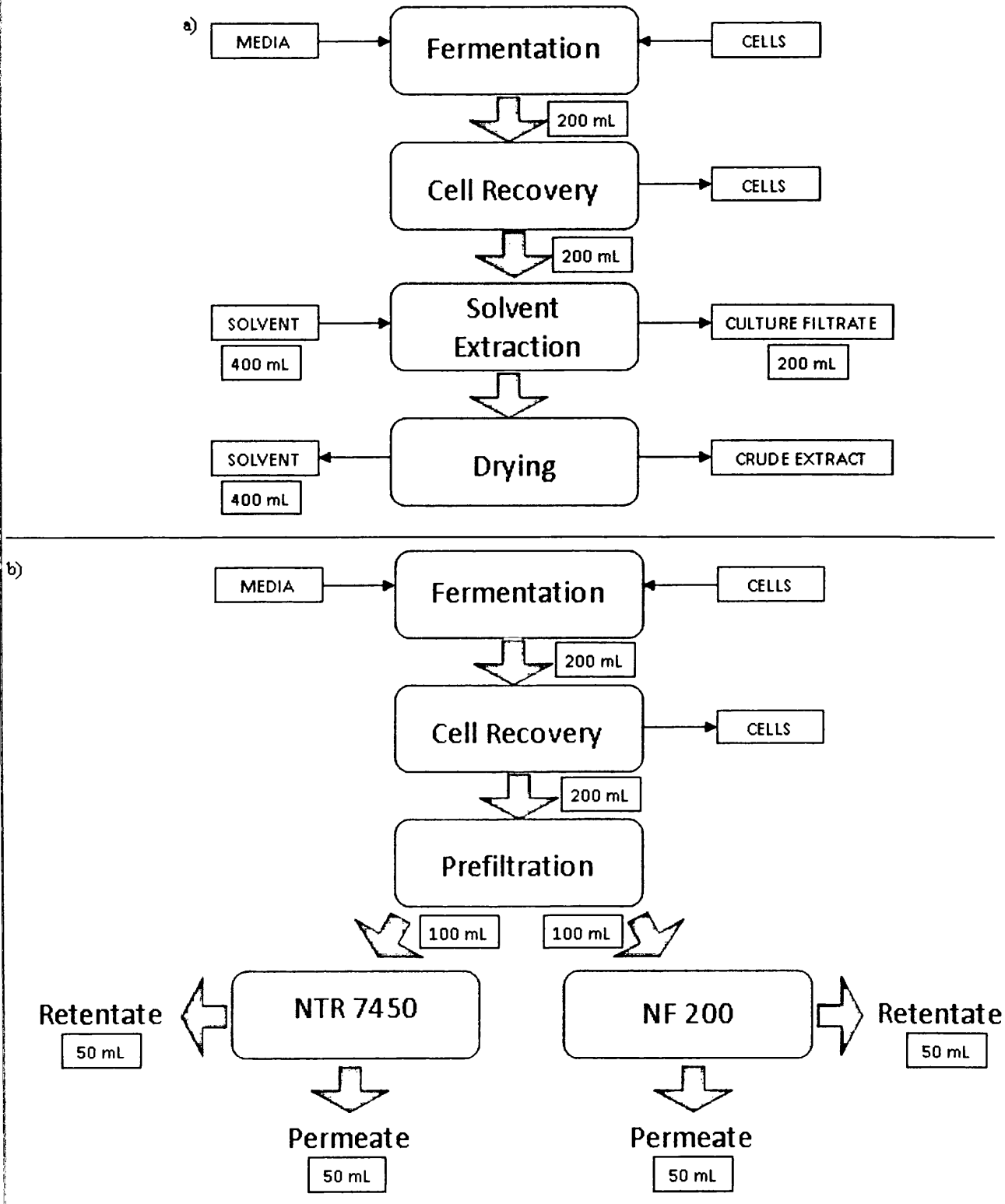

Figure 5.5: Outline and mass balance for a) the traditional process and b) the membrane process.

5.3.3 Flux performance

Table 5.1 displays the full range of strains and media used in this study as well as the respective sample number which shall be used as means of identifying the strain and media used in this chapter. Figure 5.6 displays the membrane flux performance during the prefiltration of sample 2 (4556 + Peptone) with Table 5.2 displaying the flux performance of the membrane for all the samples processed. The time taken to process 200 mL of culture filtrate ranges from 47 to 118 minutes with samples 6 and 8 respectively. The flux data presented in Table 5.2 is all presented as flux per bar to allow direct comparison between each different process. However, to account for any defects in the membrane manufacture process the flux is quoted as a percentage of the membrane clean water flux.

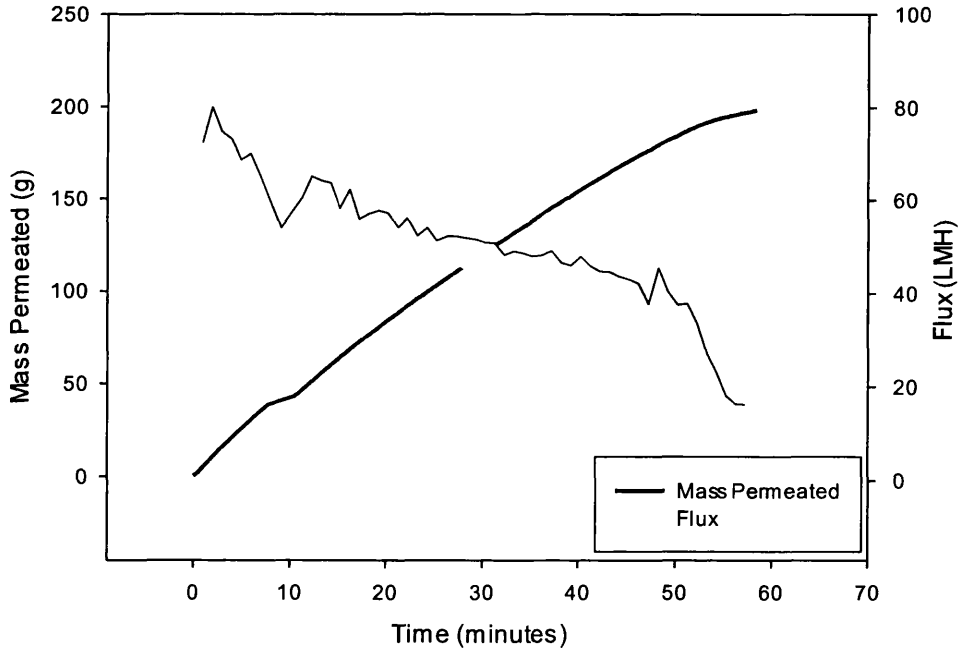

Figure 5.6: Membrane fluxes of sample 2 (4556 + Peptone) during prefiltration (Single run displayed)

Figure 5.7a and b displays the flux performance during the filtration of sample 2 (4556 + Peptone) for b) NTR-7450 membrane and c) NF200 membrane. Table 5.3 displays the flux rates for all of the samples processed by nanofiltration. The plot of flux against time for the NTR-7450 membrane shown in figure 5.7a indicated a

significant flux decline in the permeation of 50 mL of sample 2 suggesting the development of a layer of fouling material. The initial flux of sample 2 is approximately 55 LMH (20 bar) before reaching a final flux rate of approximately 22 LMH (20 bar) after the permeation of 50 mL. This pattern of flux decline was repeated with all the other samples processed by the NTR-7450 membrane suggesting that the membrane is prone to fouling by the compounds present in the process fluid. The fouling observed during the nanofiltration process when the NTR-7450 membrane was likely to initially be surface fouling as the membrane displayed a significant flux decline in a relatively short time frame. The NF200 membrane displayed very little flux decline in the same time period which therefore suggests that the NF 200 membrane was unaffected by surface fouling. The difference in pore size between the two membranes studied would suggest that in fact the NTR-7450 membrane observed pore fouling which then quickly led on to surface fouling hence the rapid and significant flux decline. The smaller pores of the NF 200 would prevent the initialisation of the pore fouling. In the case of both membranes but in particular the NTR-7450 membrane fouling may be prevented of at least reduced by operating in cross-flow mode as opposed to frontal filtration mode as studied. Despite the stirrer rotation speed being optimised for the equipment used the use of a cross flow mode helps scour the membrane surface continuously with fresh feed which reduces the size of the concentration polarisation layer that is able to develop. The fouling observed may be reversible upon cleaning of the membrane surface, however this aspect was not studied as studying membrane cleaning at a laboratory scale in frontal filtration mode is unfeasible. The development of a foulant layer upon the membrane surface would alter the rejection properties of the membrane. There is a possibility that the membrane may display a higher rejection of compounds due to the build-up of a foulant layer on the membrane surface, which may be the case in this study where the looser NTR-7450 membrane displayed similar rejection results to the NF200.

Table 5.2: Prefiltration flux rates

			Membrane Flux (LMH/bar)		
		200 mL Processing Time (min)	4000 MWCO	4000 MWCO	% of Clean Water Flux
Sample	Name		Clean	Process	
1	**4556 + Yeast**	52.25	9.36	5.45	58.23
2	**4556 + Peptone**	58.54	10.24	4.95	48.34
3	**4556 + 10:01**	88.20	9.4	3.23	34.36
4	**Arsef 23 + Yeast**	55.50	10.07	5.12	50.84
5	**Arsef 23 + Peptone**	65.15	9.01	4.34	48.17
6	**Arsef 23 + 10:01**	47.75	11.07	5.98	54.02
7	**V275 + Yeast**	95.75	12.65	3.00	23.72
8	**V275 + Peptone**	118.30	9.02	2.42	26.83
9	**V275 + 10:01**	72.70	10.04	3.92	39.04

Table 5.3: Nanofiltration Flux rates

			Membrane Flux (LMH/bar)			Membrane Flux (LMH/bar)			
		50 mL Processing Time (min)	NTR-7450	NTR-7450	50 mL Processing Time (Mins)	NF 200	NF 200	% of Clean Water Flux	
Sample	Name	NTR-7450	Clean	Process	NF 200	Clean	Process	NTR-7450	NF200
1	**4556 + Yeast**	19.75	7.01	1.30	145.25	0.28	0.28	18.6	100.0
2	**4556 + Peptone**	22.25	5.76	1.19	155.00	0.33	0.29	20.7	86.4
3	**4556 + 10:01**	23.20	2.29	1.71	102.40	0.29	0.12	74.6	42.1
4	**Arsef23 + Yeast**	14.70	6.60	1.86	239.70	0.19	0.17	28.1	91.9
5	**Arsef 23 + Peptone**	18.40	7.02	1.33	147.80	0.40	0.23	19.0	56.3
6	**Arsef 23 + 10:01**	38.50	2.84	0.76	44.75	1.12	0.84	26.8	74.6
7	**V275 + Yeast**	51.75	6.17	0.43	69.25	0.73	0.45	7.0	61.6
8	**V275 + Peptone**	28.00	3.15	0.87	36.00	2.47	0.78	27.5	31.4
9	**V275 + 10:01**	43.20	4.92	0.69	114.10	0.33	0.33	14.0	100.0

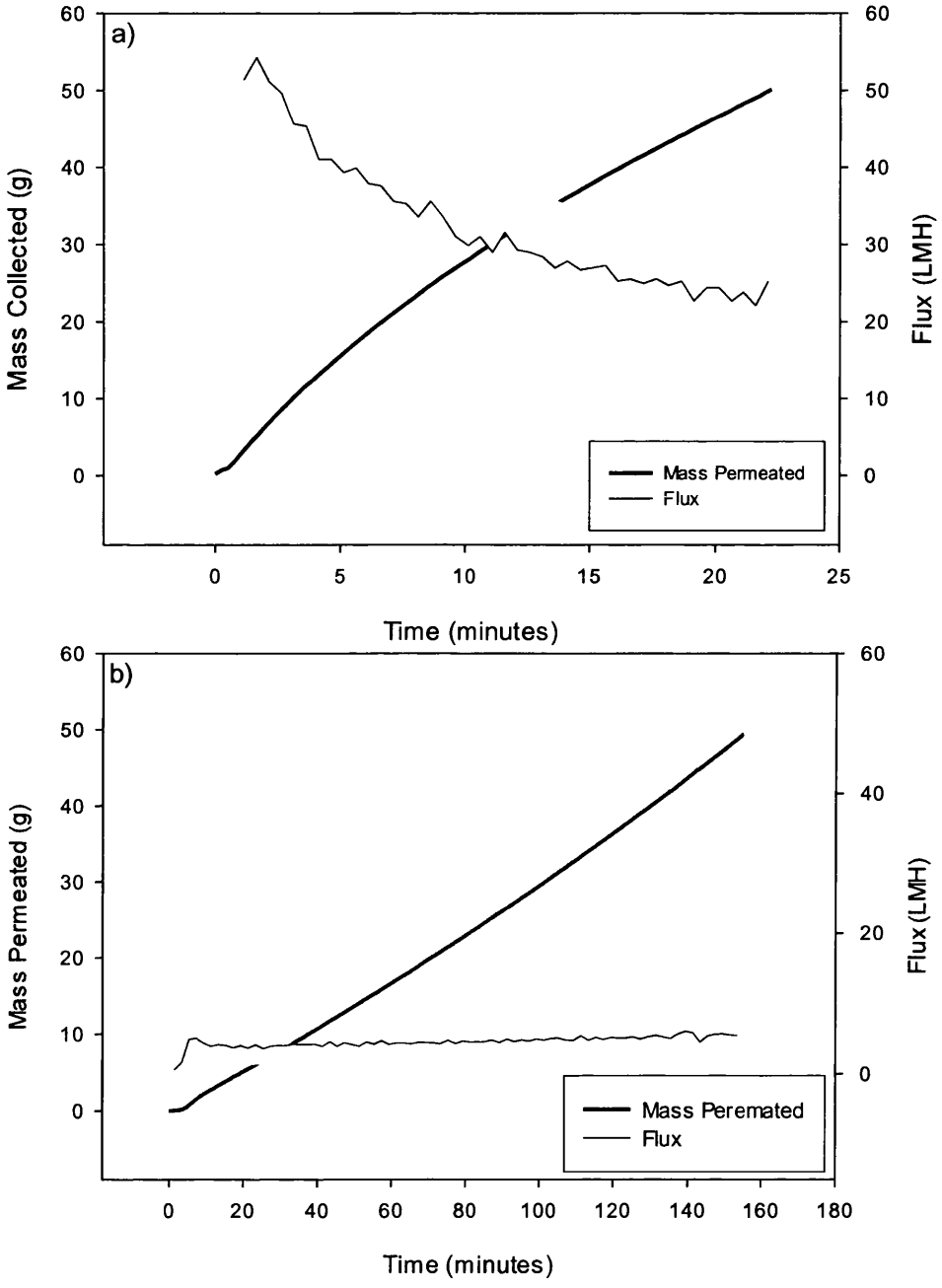

Figure 5.7: Membrane fluxes of sample 2 (4556 + Peptone) during nanofiltration for a) NTR-7450 b) NF 200 (Single runs displayed)

In figure 5.7b the flux performance of the NF200 membrane is displayed when processing 50 mL of sample 2. Interestingly there is no significant reduction in flux during the permeation of 50 mL, however the processing time is 155 minutes compared to 22 minutes when processing the same volume of sample using the NTR-7450 membrane, suggesting that the NF200 membrane is less prone to fouling when compared to the NTR-7450 membrane. This result is expected as the NTR-7450 membrane is known as a high flux NF membrane and has a higher reported MWCO and hence larger membrane pores to allow for higher flux rates. Furthermore the low MWCO (~300 Da) of the NF 200 membrane may result in pores small enough to prevent any deposition of foulants within the membrane pores and hence the maintenance of a constant flux. The flux rate of processing of sample 2 by the NF200 membrane is 5.7 LMH (20 bar) which is approximately four times lower than the lowest flux rate displayed by the NTR-7450 at similar operating conditions. The end of operation flux per bar associated with the NF200 membrane is lower than the flux per bar obtained with the NF 200 membrane in all but two cases (samples 6 and 7), however in the case of samples 6 and 7 the filtration time is quicker when the NTR-7450 membrane is used as opposed to the NF200 membrane (Sample 6: NTR-7450 - 38 minutes, NF200 - 45 minutes; Sample 7: NTR-7450 - 52 minutes, NF200 - 69 minutes), the flux decline is due to the development of a fouling layer on the NTR-7450 membrane. Overall the 50mL processing time is 5.5 times quicker when the NTR-7450 membrane is used when compared to the NF 200 membrane, furthermore the average final flux rate of the NTR-7450 membrane across the samples studied is five times greater than that of the NF 200. The experimental work undertaken used a stirrer speed of 300 rpm which has been shown to be optimal for the reduction of concentration polarisation for the equipment used. The results obtained would suggest that despite the optimisation of the stirrer there is significant deposition on the membrane surface in particular with the NTR-7450 membrane. The larger pore size of the NTR-7450 membrane compared to the NF200 membrane resulted in a shorter processing time, however, as a result of the higher flux the membrane was prone to more significant fouling, likely due to particle deposition within the pores and around the pore entrances.

In order to rationalise these findings, the flux rates were compared to each membranes clean water flux rate. The results obtained showed that the NF200 membrane sample flux rate was operating at an average of 71.5% of the clean water flux rate whereas the NTR-7450 membrane was operating at only 26.2% of the clean water flux, therefore confirming the development of a significant fouling layer. The average processing time of the NF200 membrane was 117 compared to a average processing time of 29 minutes for the NTR-7450 membrane which is four times faster, and therefore a more feasible process. Overall there is no observable pattern between media composition, strain used and membrane flux performance and suggests the flux performance is subject to vary from sample to sample. This relationship may potentially be further investigated by studying membrane flux in cross-flow mode to try and reduce the fouling experienced and by further investigating the media, strain and fermentation properties. This would be specifically undertaken by studying the fermentation for each specific strain and media. Properties such as cellular growth, optical density and media composition (media uptake) could be studied to see whether the more intricate properties of the strain and media could suggest how the membrane will perform. Furthermore, studying the effect of cross flow rates, as well as operating pressures in cross-flow mode should allow in combination with the feed (fermentation) properties the membrane fouling to be further investigated.

Figure 5.8 shows a series of SEM images of the NTR-7450 membrane substructure, in order to visualise the membrane compaction and surface deposition.

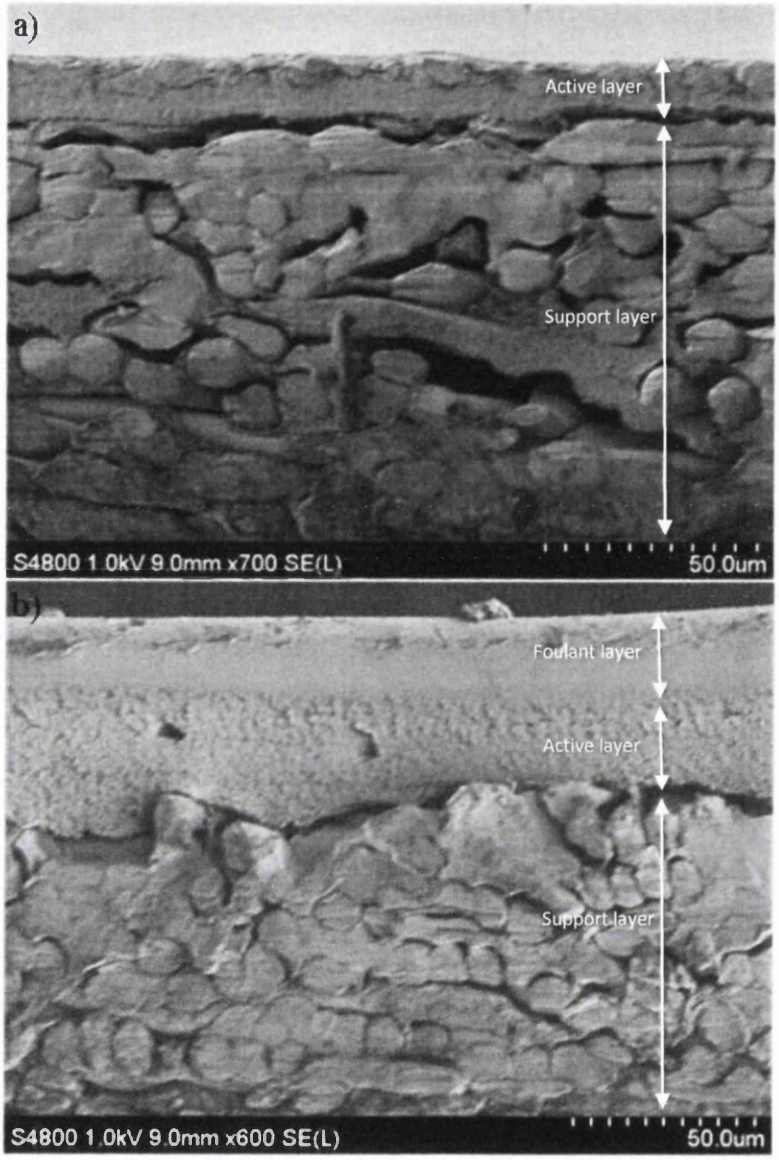

Figure 5.8: SEM Images of the NF membrane substructure, a) virgin NTR-7450 membrane, b) the NTR-7450 membrane post operation (after pre-treatment process)

Figure 5.8a shows a transverse image of the NTR-7450 membrane with the active top layer (~10 to 15 μm) highly visible and the support structure beneath. Figure 5.8b shows the same NF membrane following NF filtration after pre-treatment. The active top layer now appears to be much thicker (~50 μm), this is a result of the foulant materials impregnating on to the membrane surface. In real terms the active layer may have observed some compaction under operation; however the actual active layer thickness would not be affected by foulants, and the 'thickening' as described in a case of foulants filling the interstices between the polymers. This

blocking of the surface is most likely the primary mechanism for the flux retardation observed in all the samples studied. Notice also that the support layer of the membrane is now significantly compacted when compared to Figure 5.8a, this is normal for NF operation and is the result of the high pressure used and justifies the use of the pre-flush.

The membrane fluxes are only part of the process, in an ideal situation the membrane would exhibit high flux, low susceptibility to fouling while either fully transmitting or rejecting the compounds of interest.

5.3.4 Bioactive Compound Rejections

Table 5.4 displays the membrane rejection data for destruxin E and cytochalasin through the different stages of processing. The study also attempted to study the passage of swainsonine through the process, however the quantities present in the samples produced were too low to allow for an accurate quantification and therefore are not presented. In the process designed the rejection of destrxuin E and cytochalasin through the prefiltration process would be as low as possible and ideally zero. However, obtaining almost 100% permeation of compounds is unlikely due to the build-up of a foulant layer associated with the flux reduction noted previously which will trap some compounds. Similarly for the nanofiltration membranes a build up of a foulant layer may trap the compounds of interest however in the case of this study only half of the feed volume is permeated in order to obtain both retentate and permeate fractions.

Table 5.4: Observed rejection of fungal metabolites during processing with membrane process

		Destruxin E (Rejection %)			Cytochalasin (Rejection %)		
Sample	**Name**	**Prefiltration**	**NTR-7450**	**NF 200**	**Prefiltration**	**NTR-7450**	**NF 200**
1	**4556 + Yeast**	3.2	72.4	81.8	3.1	78.2	89.5
2	**4556 + Peptone**	29.1	70.0	79.0	25.3	74.6	100.0
3	**4556 + 10:01**	4.6	9.8	29.1	0.0	100.0	38.3
4	**Arsef 23 + Yeast**	8.5	76.3	76.7	4.5	89.1	89.6
5	**Arsef 23 + Peptone**	21.0	70.5	76.7	11.2	87.4	92.8
6	**Arsef 23 + 10:01**	35.7	66.5	63.7	23.3	100.0	82.0
7	**V275 + Yeast**	9.0	12.6	13.2	16.7	100.0	100.0
8	**V275 + Peptone**	0.0	38.3	49.2	0.0	100.0	100.0
9	**V275 + 10:01**	28.4	8.8	6.4	37.3	100.0	100.0

The rejection results for destruxin E and cytochalasin are displayed in Table 5.4. The prefiltration stage obtained an average rejection of 15.5 and 13.5 % for destruxin E and cytochalasin respectively over the whole range of samples studied. Interestingly sample 8 (V275 + Peptone) displayed a 0% rejection for both destruxin E and cytochalasin, suggesting total transmission of the compounds. A similar pattern is not observed for the other samples containing the strain V275 with for example sample 9 (V275 + 10:01) displaying a rejection of 28.4 and 37.3 % for destruxin E and cytochalasin respectively, therefore suggesting rejection results are dependent on each individual fermentation. The varying rejection values are dependent on the concentrations of compound in the feed at each stage, a theory that would explain the varying rejection values over the range of samples studied. In general, the values obtained for the prefiltration are overall acceptable. The rejection values obtained for samples 2, 6 and 9 are a higher than desired, although in the worst case (sample 9) more than 60 % of the cytochalasin is permeated.

The average rejection results for destruxin E and cytochalasin were 47.2 and 92.1 % respectively for the NTR-7450 membrane and 52.9 and 88.0 % respectively for the NF200 membrane. Overall approximately 50% of destrxuin E was rejected by the nanofiltration process regardless of membrane selected with the NF 200 membrane rejecting a slight higher amount as would be expected, due to the smaller reported MWCO. However, in the case of cytochalasin the average rejection is approximately 90% over the nanofiltration process, suggesting a very efficient separation. Interestingly the average cytochalasin rejection observed was higher with the NTR-7450 membrane (92.1% NTR-7450, 88% NF200), this is unexpected as the reported MWCO of the NF200 membrane is lower smaller than that of the NTR-7450. The proximity of the results suggests that the reported membrane MWCO for both membranes are possibly incorrect, however based on the results observed the reported MWCO of the NTR-7450 is believed to be an over-estimate. The rejection results obtained for both membranes are varied, exhibiting a similar pattern to the prefiltration stage where rejection values are dependent on feed properties such as concentration. The optimum results were obtained in the processing of sample 8 (V275 + Peptone) where the prefiltration stage resulted in 0% rejection of both

cytochalasin and destruxin E. The destruxin E rejection by the NTR-7450 and NF 200 membranes was then 38.3 and 49.2 % respectively, however the rejection of cytochalasin was 100 % for both membranes. The nanofiltration rejection results obtained for cytochalasin are much less varied that those obtained for destruxin E. For example sample 9 (V275 + 10:01) displayed a destruxin E rejection of 8.8 and 6.4 % for the NTR-7450 and NF 200 membranes respectively. These values are lower than expected, and could possibly suggest a damaged or incorrectly installed membrane however the filtration runs did not coincide with an increase in membrane flux as would be expected should the membrane be damaged.

The observed rejection values obtained are varied when compared to the theoretical rejections predicted in section 5.2.3. The observed rejections are lower when compared to the theoretical rejections of the membranes used. This is due to the observed rejections reported not accounting for effects such as concentration polarisation, fouling and the effect of feed concentration. Furthermore, the values obtained from the theoretical correlations predict the separations based on model solutions, however, in the case of a complex biological solution such as this one there are a number of other interactions occurring simultaneously which affect the separation performance. The average observed rejections for destruxin E and cytochalasin were 47.2 and 92.1 % respectively for the NTR-7450 membrane and 52.9 and 88.0 % respectively for the NF200 membrane. The theoretical real rejections of both bioactive compounds studied for both membranes was 100%. This significant difference between these values may be attributed to the effect of fouling, concentration polarisation, mass transfer and the fact that a membrane MWCO is not a single finite value and that there is often a significant pore size distribution.

In terms of process design the process used in this experimental work may be improved through changing the membranes and equipment used. The work undertaken in the fungal nanofiltration study used one membrane in order to obtain a rejection value, however in reality multiple membrane modules may be used at a pilot and industrial scale which may simultaneously increase rejection while reducing fouling. The main benefit of using multiple membranes in series

would be the increased rejection which would allow looser membranes to be used in order to achieve the same overall system rejection. The use of looser membranes would give a higher permeation rate and therefore a faster processing time (higher production rate). The use of multiple modules in series is an avenue that must be explored when increasing the scale of a membrane process particularly as production rate is key to the overall success of a membrane process.

Overall the membrane based separation and purification process has been shown to be feasible for the filtration of fungal material. The prefiltration stage implemented has shown to allow permeation of the compounds of bioactive interest prior to the nanofiltration stage being used to retain the compounds. The prefiltration results obtained using the 4000 MWCO membrane are acceptable however could be further optimised by selecting a membrane with a slightly larger MWCO to allow greater permeation of the compounds. Furthermore the effect of cross-flow filtration should be investigated to help reduce fouling and subsequent flux decline. The two nanofiltration membranes studied in this work obtained comparable rejection results, however the membrane flux obtained using the NTR-7450 was much greater than that of the NF200 membrane. Taking into account the rejection and flux results the shorter processing time of the NTR-7450 membrane in producing a similar product would suggest that the NTR-7450 membrane is superior for the separation and purification of fungal culture filtrates. In the case studied the aim was to produce an extract of bioactive compounds using nanofiltration. Therefore a high percentage rejection combined with a high flux (fast production rate) would be the optimal operating conditions. The similar observed rejections of the two membranes studied would make selecting between the two membranes very difficult, however the higher processing fluxes obtained with the NTR-7450 membrane results in the selection of the NTR-7450 membrane from the two studied as the optimum for this application, despite the levels of fouling observed. The process requires much further optimisation before nanofiltration may be considered truly feasible for the purification of bioactives from fungal sources, mainly due to the flux decline observed while processing.

5.3.5 Product bioactivity assays

The fractions obtained by the two processing methods (Solvent extraction, Membrane Process) were tested as bio-control agents. The results obtained are shown in Figure 5.9.

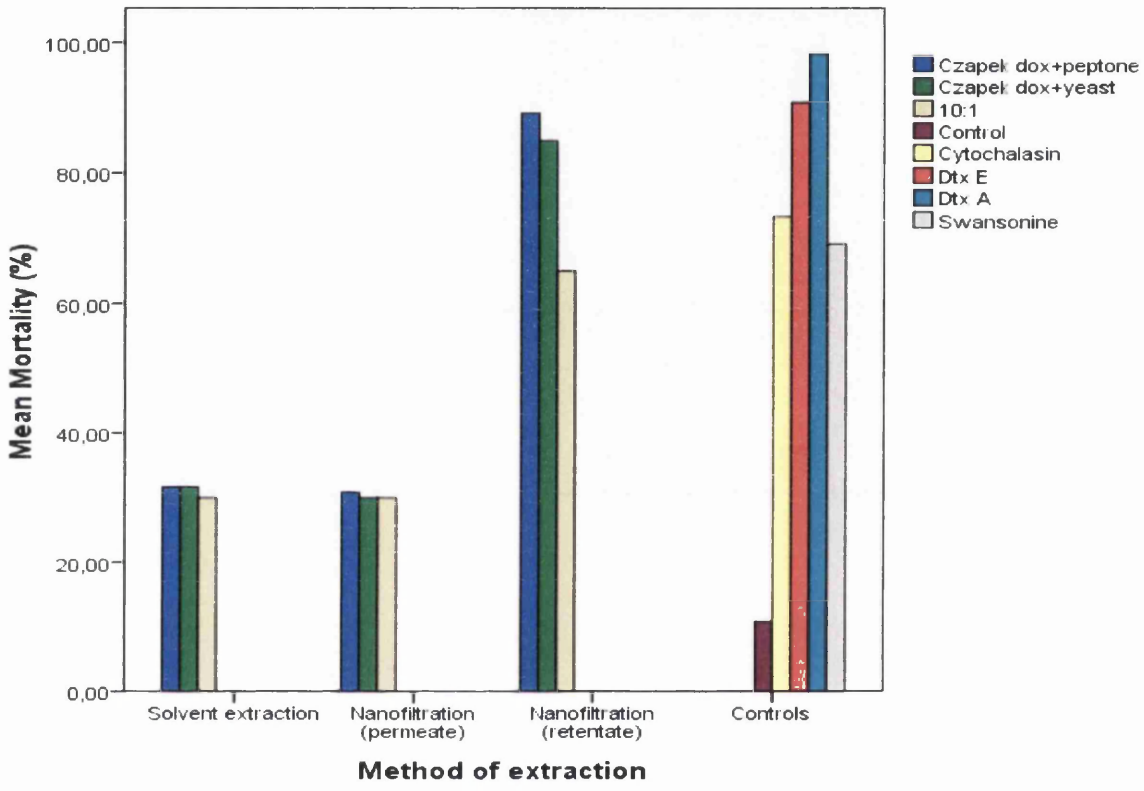

Figure 5.9: Mosquito (Culex) mortality when treated with various extracts of strain ARSEF 4556 (NTR-7450 membrane results only shown).

The mortality results obtained using the solvent extraction samples were approximately 30% for all the ARSEF 4556 strain with all three medias studied. Interestingly similar results were obtained when the NTR-7450 membrane permeate was used again displaying approximately 30% mortality. When the NTR-7450 membrane retentate was tested the resulting mortality was found to be over two times greater for all three media of the 4556 strain, suggesting the presence of higher quantities of the bioactive compounds or the presence of synergistic ratio of compounds to bring about the excellent assay response. The highest mortality of 90 % was found with sample 2 (4556 + peptone) followed by sample 1 (4556 + Yeast)

and 3 (4556 + 10:01) with 80 and 65% mean mortality respectively. A similar assay response was observed for the other samples studied however are not reported in this thesis. The results obtained suggest that the membrane based process not only maintains bioactivity but also may enhance bioassay response. This observation may be dependent on the gentle membrane process maintaining bioactivity that may otherwise be damaged by the solvent extraction process or due to the membrane process purifying compounds based on size that may otherwise be lost when separated on polarity. In order to investigate this further, the product of the nanofiltration process should be subjected to the solvent extractions process and the resulting extract should be re-tested in against the nanofiltration product to compare and alterations in product bioactivity.

5.4 Conclusions

The membrane process introduced for the recovery of small potentially bioactive compounds from fungal fermentation liquors is feasible, producing a viable alternative to the traditional separation process. The results obtained suggest the isolation additional compounds when compared to a traditional solvent extraction based recovery process. Moreover, the suggested membrane process removed the need for the use of solvents which has led to several advantages; namely, an non-bias separation based on solvent polarity as the membrane process is predominantly size based only, improved safety in the laboratory as flammable (potentially explosive) materials have been removed, no operator exposure to solvents, improved process recovery and less environmental burden in terms of effluent quantity. All of which lead to a more robust and sustainable operating philosophy. The membrane equipment will have an initial start-up capital, however, the cost savings from reduced waste materials and removal of solvent inventory will quickly recover this initial investment at the laboratory scale and will lead to cost savings in the short to medium term.

This study has incorporated two nanofiltration membranes following a ultrafiltration pre-treatment process for the isolation of these compounds. The prefiltration stage was deemed necessary to help alleviate the significant fouling

observed through direct-NF processing, combined with initial results suggesting that not all of the bioactive compounds were being permeated. Therefore two NF membranes were studied for the purification of these compounds aiming to produce a pure bioactive fraction. The prefiltration stage was a successful implementation allowing transmission of over 85% of the bioactive compounds studied at a relatively high flux rate, suggesting a feasible process. The two NF membranes studied a great variation in the membrane flux performance, with the NTR-7450 displaying short processing times although experiencing significant flux decline with processing time. The NF200 membrane was much less susceptible to fouling displaying no flux decline, however the flux rate was very low leading to very long processing times, and therefore resulting in an unfeasible process. The rejection of destruxin E and cytochalasin by the two NF membranes was studied, finding that the rejections were fairly similar for the two membranes, a surprising result considering the difference in MWCO of the membranes, suggesting the MWCO of the NTR-7450 is over-estimated when quoted as 1000. Overall the NTR-7450 membrane outperformed the NF200 membrane when considering similar rejection results were obtained at a much higher flux rate and lower processing time.

Furthermore, the fractions produced through this experimental work were subjected to a number of bioassays, testing efficiency as a pest control agent as well as attempting to discover any novel compounds that may be present by not applying a polarity based separation mechanism. The results obtained from the mosquito assay showed that the mosquito mortality was two times greater using the NF retentate as opposed to the NF permeate and the solvent extraction product (which had similar assay mortality results), suggesting that the nanofiltration retentate contained other bioactive compounds which are not extracted by solvent extraction.

Finally, the membrane process described in this work has shown promise for the recovery of small bioactive compounds from fermentation liquors. While the process is still under development and requires optimisation, the results obtained

from this study demonstrate that a membrane process is entirely feasible for this operation at the laboratory scale. The membrane process potentially offers the scientist or engineer improved bioactive material recoveries with lower material inventories, lower environmental, health and safety risks and lower overall operational cost, combined with the potential of discovering compounds otherwise missed by separating on a size basis.

6.0 Generating a Value Stream from Potato Peel Waste

The work contained within this chapter aims to assess the potential of membrane technology for the recovery of a bioactive compound from waste potato peel. The study comprises of an initial storage study which aims to examine compositional changes in the potato, hence quantities of bioactive available, with storage time and conditions. This is followed by an extraction study which aims to examine the extraction profile of the bioactive compound prior to separation and purification using membrane technology on both laboratory and pilot scales.

6.1: Composition and Calystegine Extraction Study

6.1.1 Introduction

Plants, fungi, and bacteria, provide a virtually unlimited source of novel compounds for a wide range of health benefits, either in pure form or as standardised extracts. Natural products have long been recognised as an important source of therapeutically effective medicines. Natural products are typically classified as organic materials derived from animal or plant extracts. Biopharmaceuticals represent a significant and growing proportion of the overall pharmaceutical market and the sales of biologicals were valued at $94 billion in 2007 (Walsh, 2010). The therapeutics derived from natural products is generally classified as macromolecules or small molecules.

Over the years humans have made enormous use of natural compounds; the latest version of the *Dictionary of Natural Products* (DNP; http://dnp.chemnetbase.com) contains over 250,000 entries. Currently only a small proportion of natural sources have been exploited and released of their full potential, therefore there is ample opportunity for further progress (Cos et al. 2006). The World Health Organisation (WHO) estimates that more than 80 % of the world's population relies on traditional medicine from natural sources for their primary healthcare needs (Sasidharan et al. 2011). The chemical complexity found in natural products can be very difficult to synthetically replicate and natural products often have been largely ignored by pharmaceutical companies due to the lack of assurance regarding a reliable extracted supply of material. Despite the lack of interest, approximately

50% of all small molecule drug leads discovered between 2000 and 2010 were derived from natural products (Newman and Cragg, 2010).

Potatoes (*Solanum tuberosum* L.) are one of the most important food sources worldwide, along with wheat, rice and corn. Their versatility and energy richness produces more dry matter, protein and minerals per unit area in comparison to cereal crops (rice, wheat, barley) (Ezekiel et al. 2013) and potatoes are grown in more countries than any other crop except maize (Lister and Munro, 2000). The potato, originally from South America, appeared in Europe late in the sixteenth century and since has become a staple component of many European meals (Howard, 1970; Weichselbaum, 2010; Murniece et al. 2011) often served as a starchy side dish. The nutritional profile of potatoes have beneficial effects on human health, the rich source of energy combined with health promoting phytonutrients such as phenolics, flavonoids, folates, kukoamines, anthocyanins and carotenoids make potatoes highly desirable in the human diet (Katan and DeRoos, 2004; Ezekiel et al. 2013) and current worldwide potato consumption stands in excess of 300 million tonnes per annum (Smith et al. 1996; Keijbets, 2008; Lin Ek et al. 2012). Fresh potato consumption used to be the major part of potato utilisation, although recent years has seen this level of consumption continually decrease, particularly in developed countries with increasing quantities of potatoes processed into value-added foods stuffs to meet the demand of the fast food and convenience food industries (Schieber et al. 2001; FAO, 2008; Schieber and Saldaña, 2009; Bates et al. 2010). Statistics show UK consumption has decreased from 104 to 95 g/day between 2000 and 2009 (Riley et al. 2010), however, the downward trend of potato consumption opposes the increase in the quantity of starchy carbohydrates being consumed.

Potato tuber composition is dependant on many factors, including species, soil type, climate, growing time, harvesting method, storage conditions and storage time (Ashby, 1905; Kumar et al. 2004; Knowles et al. 2009; Frančáková et al. 2011). Potato industry by-products may be classified into three major types: cull potatoes, whole potatoes not destined for human consumption and potato processing water. These waste streams are obtained from the manufacture of potato based value

added foods and liquid waste from processing one metric tonne of raw potatoes results in a reported 8,000 to 28,000 litres of waste water (Charmley et al. 2006). Potato peel wastes (PPW) and potato industry waste waters are a low value, high volume waste from industrial potato processing (Arapoglou et al. 2009). The losses generated from peeling potatoes range from 15 to 40% of mass dependant on the procedure used, i.e. steam, abrasion or lye peeling (Schieber et al. 2001), with Charmley et al. (2006) estimating 40 to 50 % of potatoes produced being unsuitable for human consumption.

Potato peel represents a major percentage of the processing waste. Wet peels are prone to rapid microbial spoilage (Schieber et al. 2009) therefore quick and efficient disposal is important. Potato peel has previously been studied for antioxidant content (De Sotillo, 1994; De Sotillo, 1994a; De Sotillo, 1998; Al-Weshahy, 2013) along with a review of pharmacologically interesting compounds by Schieber and Saldaña (2009). Calystegines, a class of water soluble nortropane alkaloids, were first discovered in 1988 from root cultures of the non-food plant *Calystegia sepium* (bindweed). The structure of the most common calysteines (A_3 and B_2) found in potatoes is shown in Figure 6.1.1.

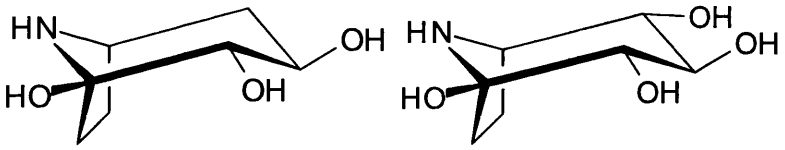

Figure 6.1.1: The main calystegines found in potatoes are A_3 (left) and B_2 (right)

Since first discovery, calystegines have been found in a number of other plant families including Convolvulaceae *(Convolvulus arvensis)* and of interest in this thesis Solanaceae (Molyneux et al. 1993; Schimming et al. 1998; Bekkouche et al. 2001). Calystegines contain either three (calystegine A), four (calystegine B) or five hydroxyl groups (calystegine C). Due to their structural similarity to sugars and iminosugars, calystegines exhibit potent and specific glycoside inhibition specifically α- and β-glucosidases and α–galactosidase (Watson et al. 2001) and therefore make interesting compounds for research on new bioactives (García-Moreno et al. 2004). Interestingly calystegines have been significantly less explored when compared with

other iminosugar structures, with the structural basis of calystegines glycosidase inhibition only partially established (García-Moreno et al. 2001).

Of the Solanaceae plants, sweet peppers, eggplants and sweet potatoes are the food plants containing the highest total amount of calystegines (up to 80 mg/kg fresh weight) (Andersson, 2002). Potatoes (*Solanum tuberosum*) usually contain less of these compounds – approximately 1 to 10 mg/kg of calystegine A_3, B_1, B_2 and C_1 (Andersson, 2002) but can contain over 450mg/kg fresh weight in the skin (Friedman et. 2003). Calystegines have been reported in potato leaves and skin (Keiner and Dräger, 2000); however, the highest concentrations were measured in sprouting potatoes (Dräger et al. 1995; Molyneux et al. 2007).

Calystegine B_2, B_3, and B_4, have been synthesised artificially from D-glucose, D-galactose and D-mannose respectively (Boyer and Hanna, 2001; Skaanderup and Madsen, 2003) with Boyer and Hanna (2001) suggesting that other calystegine may be synthesised using a similar approach. The synthetic manufacture of calystegines involves the use of a diverse range of organic solvents, chemicals and catalysts and therefore the likely high costs and environmental hazard associated with the process would make it unfeasible. Furthermore, the abundance of waste potato peel lends an ideal feedstock for the isolation and purification of calystegines. Potato peels are known as a zero value waste (Arapoglou et al. 2009). Traditionally potato peel wastes (PPW) are applied to agricultural land as a disposal medium, providing an excellent source of nitrogen, with prolonged application leading to increasingly fertile soils. Peel disposal in such a manner provides and effective method of reusing nutrients that would otherwise be wasted, and subsequently provides a relatively easy method for PPW disposal (Radunz et al. 2003; Hung et al. 2004). Recent years have seen the emergence of other methods for PPW reuse, such as animal feed (Tawila et al. 2008), heavy metal removal from industrial effluent (Aman et al. 2008), bioremediation (Okeke and Frankenberger Jr. 2005; Maleki et al. 2013) and ethanol production (Arapoglou et al. 2010).

There is also a drive from many of the worlds markets for personal care products, cosmetics and pharmaceuticals derived from natural products rather than synthetic

origin (Wijesinghe and Jeon, 2011). Thus, based on the successes of the past, the current trends in consumerism and the failings of combinatorial chemistry, natural products will most certainly play a key role in the development of the next generation of medicines and therapeutics.

Green potatoes should not be consumed as glycoalkaloids levels are elevated in greening areas of the tuber (Jensen, 2008) and several cases of human poisoning being described by Friedman and McDonald (1997). Typical symptoms of human poisoning include nausea, vomiting, diarrhoea and abdominal pain (Mensinga et al. 2005). Glycoalkaloids are normal constituents of conventional potato varieties and contribute in small amounts to typical potato flavour (Knuthsen et al. 2009). However, in large quantities glycoalkaloids lead to a bitter tasting potato and cause poisoning in humans. All *Solanaceous* plants such as tomatoes, peppers and in particular potatoes synthesise a variety of compounds which serve as a natural defence mechanism against fungi, viruses, bacteria and insects (Friedman, 2004).

Food production and food processing from agricultural products generates large quantities of waste material annually. Global production of potatoes stands in excess of 300 million tonnes per annum (Keijbets, 2008; Lin Ek et al. 2012) with a value of 324 million tonnes (MT) reported for 2010, (Van Dyk et al. 2013). However, despite the worldwide potato production increasing, recent years have seen the consumption of fresh potatoes, particularly in developed countries, decrease. Increasing quantities of potatoes are being processed into value added products to meet the demand of the fast food and convenience food industries (FAO, 2008; Schieber and Saldaña, 2009; Bates et al. 2010). Peel waste from processing estimated to be 15 to 40 wt% (48 – 129 Million Tonnes) depending on processing method. Therefore, potatoes (*Solanum Tuberosum)* are an ideal feedstock for the extraction and purification of calystegines.

The qualitative and quantitative studies of bioactive compounds from potatoes are reliant on efficient selection of extraction methods. Extraction is the first step in the drug/pesticide/nutraceutical discovery process from natural materials. A large variety of extraction methods have been used and are often based on a selected

property of the compound of interest (e.g. solubility, polarity...etc.). Basic extraction operations include washing, drying of plant material or freeze drying, and grinding to obtain a homogeneous sample and to improve extraction kinetics and efficiency. General extraction procedures include solid-liquid (or liquid-liquid) extraction, infusion and maceration; modern more advanced techniques such as microwave assisted extraction, ultrasound assisted extraction, super critical fluid extraction and pressurised liquid extraction have been developed (Brusotti et al. 2014). The large scale extraction of calystegines would allow the true benefit of the compounds to be explored; however using very large volumes of organic solvents at an industrial scale is undesirable for safety, environmental and economical reasons. Calystegines display the potential to inhibit mammalian glycosidases. The glycosidase inhibition exhibited by calystegines has led to the discussion of potential applications such as anti-diabetic, anti-viral, anti-biotic, and immune stimulation. Therefore potential applications are areas such as medicines and therapeutics or as a natural food preservative (the food preservative market alone worth over $2 Billion (Marketandmarkets.com, 2014)). Thus far, studies into calystegines have used small laboratory scale extractions using organic solvents in order to investigate the properties of calystegines whereas this study attempts to investigate the maximisation of calystegine concentration prior to large scale extraction without any organic solvents. The exclusion of organic solvent from the recovery process will allow the compound to be used for a variety of purposes without the issue of solvent contamination.

In the past, separation of calystegines has been undertaken on a small lab scale basis. Calystegines are usually extracted from both fresh and dried plant material. Various extraction methods reported in the literature are shown in Table 6.1.1.

Table 6.1.1: Calystegine extraction methods reported in the literature

Plant materials	Mass (g)	Plant material state	Extraction Solvent	Purification method	Reference
Solanaceous species	20	Air-dried (Powdered)	Water:Methanol (80:20)	Ion Exchange (Dowex 50W X8-400 H^+; Amberlite IRA-400 Cl^-)	Bekkouche et al. (2001)
Solanum Tuberosum	1	Lyophilised (Powdered)	Methanol:Water (4:1)	Ion Exchange (Dowex AG 50W X 8)	Friedman et al. (2003)
Solanum Tuberosum	1 - 5	Lyophilised		Ion Exchange (Cationic - I LAB)	Keiner and Dräger (2000)
Convolvulaceae	10	-	Methanol:Water (1:10)	Ion Exchange (Dowex 50W X8)	Schimming et al. (1998; 2005)
Edible fruits and vegetables	-	Fresh	Methanol or Ethanol	Ion Exchange (Amberlite IR-120B)	Asano et al. (1997)
Convolvulaceae Solanacae	550	Dried	Hot water	Ion Exchange (Amberlite IR-120B; Amberlite CG-50; Dowex 1-X2)	Asano et al. (2001)
Calystegia sepium	1 - 3	Fresh Dried	Methanol	Ion Exchange (I LAB)	Scholl et al. (2003)
Erythroxylum	50 - 100 (mg)	Dried		Ion Exchange (Cationic -I LAB)	Brock et al. (2005)

Potatoes	100 - 200 (mg)	Dried	methanol	Ion Exchange (I Resin)	Kvasnička et al. (2008)
Solanum Tuberosum	-	Lyophilised (Powdered)	methanol	Ion Exchange	Griffiths et al. (2008)
Solanum Tuberosum	100 (mg)	Lyophilised	Methanol:Water (1:1)	Ion Exchange	Richter et al. (2007)

The composition-storage work in this sub-section is critical to the understanding of the separation required. The primary objective of this work was to evaluate compositional changes in potato peel stored in four conditions over an eight week storage period, paying particular attention to the calystegine content and aiming to find optimal storage conditions in order to maximise calystegine concentration. The storage conditions selected aim to reflect typical industrial storage conditions and the effect these conditions have on the potato peel composition. The effect of storage time is also investigated in order to see whether storage conditions affect the quantity of calystegines in the potato peel. In addition, an extraction study was undertaken to determine the rate of release of various compounds, including proteins, sugars and calystegines, from both whole potato and waste potato peel into an aqueous phase as a precursor to purification with a novel separation methodology.

6.1.2 Materials & Methods

Raw material

Potatoes (Variety: Accord) were purchased locally (S&E Ford (Puffin Produce), Pembrokeshire, UK) in early July, to ensure freshness and potatoes free from any additives and preservatives. The potatoes selected for the storage study were approximately equal in size to minimise any errors, with extremely large or small potatoes not being included in the study. An attempt was made to select potatoes that were round and crevice free as far as reasonably possible. For this study potato peel is defined as whole potatoes peeled at the beginning of the study and stored as peel for the duration of the study. Whole potato is defined as potatoes stored as

whole tubers and then peeled immediately before lyophilisation and composition analysis. All potato tubers were rinsed in clean water to remove any remaining soil and wiped to ensure no surface water remained. In order to prepare the peel samples for analysis all potatoes were hand-peeled to an approximate depth of 2mm using a hand peeler.

Tuber samples were prepared by carefully storing both whole tuber and peel only samples at four conditions, namely: cold-dark (5±3°C, no light), cold-light (5±3°C, natural daylight), Warm-dark (30±5°C, darkness), warm-light (30±5°C, natural daylight). The cold-dark samples were placed in a cardboard box with ventilation holes covered by tissue paper inside a fridge. The cold-light samples were stored in a glass fronted fridge on a tray to allow exposure to natural daylight (Only the potatoes were stored in the fridge). The warm-light samples were stored on a tray in a warm constant temperature room and exposed to natural daylight. The warm-dark samples were stored in a plastic container with holes in the same warm room. The conditions were designed to represent the storage conditions that may be found on an industrial scale, where waste potatoes and peel may be either kept in an environment where the temperature is not controlled (i.e. ambient) or in chilled storage (usually 5°C). Similarly the light duration and intensity were not monitored, however as the studies were performed in July the light exposure would be greater than 12 hours (region of 12-16 hours).

Extraction study

In order to calculate the extraction profile of the different compounds, 100 g of fresh potato peel was added to 1 litre of ultra pure water (10 kg in 100 litres for calystegine analysis to increase the total mass of calystegines present). Two peel cuts were used for the extraction study, hand peeled strips and chopped peel (chopped peel was cut into approximately 1 cm^2 pieces, all cutting was performed with same equipment and by the author). The extractions were conducted at 20 °C and at four stirrer speeds (0, 60, 120 and 180 rpm) with 10 mL samples taken at 1, 3, 5, 10, 20, 30, 60 and 180 minutes. The apparatus (shown in figure 6.1.2) consisted of a 1 litre conical flask placed beneath a pillar stirrer with the impeller

(25 mm axial impeller) immersed in the flask 25 mm from the base. Samples were taken from the 900mL mark on the beaker. For the experimentation an axial flow impeller was selected as they are known to help keep solids in suspension and therefore promote thorough mixing when solids are present. The impeller selected prevented any solids potato peel from settling on the bottom of the beaker. The experiments were compared to the water values when there were no potatoes present as a control. The samples were immediately analysed for protein and total sugar content using the analysis methods described in chapter 3 and additional samples were frozen at -40 °C until calystegine analysis could be performed by GCMS.

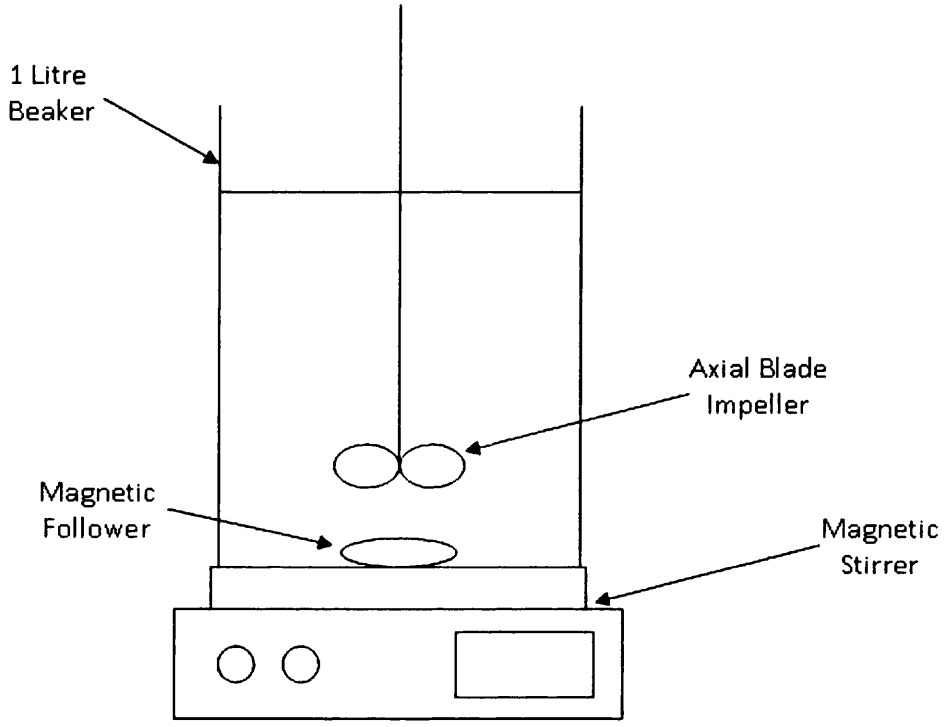

Figure 6.1.2: Diagram of potato extraction equipment

Calystegine Analysis

Calystegine analysis is easier if the samples are first purified using ion exchange to reduce levels of sugars. 1g of freeze dried material was added to 20 mL of ultra-pure water and extracted for 2 hours, vortexing every 30 minutes. The resin used in this study was the Amberlite IR-120+ (Rohm and Haas UK Ltd, UK), a strongly acidic cationic exchange resin based on a polystyrene-divinylbenzene copolymer with a

sulfonic ($R\text{-}SO_3^-$) active group. The resin was pre-treated with two column (12 cm long with internal diameter 2 cm, bed height 6 cm) volumes of 2M HCl (Fisher Scientific, Loughborough, UK) left to soak for 15 minutes, followed by 10 column volumes of ultra pure water. 10 mL of the samples were then loaded onto the column and left to stand for 10 minutes. The sample was then released from the column in drop-wise fashion followed by 10 column volumes of ultra pure water into a waste container. The bound components were then released and collected by two column volumes of 2M Ammonium Hydroxide (Fisher Scientific, Loughborough, UK) into a round bottom flask and dried using a Rotavapor R-210 (Buchi, Oldham, UK). The columns were rinsed with ultrapure water before regeneration with 2M HCl for 15 minutes before processing further samples. The dried samples were re-dissolved in 3mL of water before being transferred to pre-weighed vials prior to freeze drying for 24 hours. The samples were then analysed by GC-MS as trimethylsilylderivatives (Pierce TriSil) with 25 μg of authentic castanospermine (PhytoQuest, UK) added as internal reference (the method is described in the patent PCT/GB2003/000906 - Process for monitoring the quality of a herbal medicine, WO 03/074147 A1).

6.1.3. Results & Discussion

Potato Composition

Potato appearance & Quality

The appearance and quality of the potato was monitored over the total period of the study. The fresh potato tubers when purchased had a very thin, delicate skin layer, which was easily removed if abrasively scrubbed indicating an immature tuber or an early season variety. The thickness of the potato skin increases on plant maturation increasing tuber protection from spoilage by microorganisms and prevention of tuber dehydration (Lulai and Freeman, 2001; Buono et al. 2009, Neubauer et al. 2013). The skin on the whole potatoes thickened with progressing time in all storage conditions (visual inspection only). Initially the potatoes when purchased were firm, although when stored in the differing conditions the firmness of the potatoes varied significantly. In all cases the firmness of the whole potatoes

decreased, although this phenomenon was observed to occur most rapidly in the potatoes stored in warm and light conditions. The whole potatoes stored in cold and dark conditions remained firmest at the end of the period studied and is a similar finding to that previously reported by Burton (1969). Whole tubers stored in warm and dark conditions remained fairly firm throughout the study although developed white patches of microbial growth towards the end of the study. This was believed to be due to the warm dark conditions leading to high storage humidity resulting in an excellent growth environment for spoilage organisms. The whole tubers exposed to light during storage displayed some greening towards the end of the storage period; the tubers stored in the cold conditions displayed some slight greening, whereas the potato tubers stored in the warm light conditions displayed significant and severe greening, suggesting elevated glycoalkaloid concentrations (Friedman and Dao, 1992).

The peel when freshly harvested and stored was soft and malleable. In contrast to the whole potatoes, the peel appearance and texture altered quickly with storage time. However, similar to the whole potato, the quickest change was observed with the peel stored in warm and light conditions. All the stored peel eventually became darker and more brittle regardless of storage conditions. Potato peel when stored dries quickly due to the large surface area and rapid decrease in moisture is expected in warmer conditions. As with whole tubers, the peel stored in warm and dark conditions developed some spots of microbial growth.

Composition Analysis

Water Content

Potatoes are known to be approximately 80 % water (Burlingame et al. 2009; Schieber and Saldaña, 2009). Figure 6.1.2 shows the water content over the eight week storage period. The initial results showed that the potatoes used had approximately 83 % water. The whole potatoes when stored over an eight week period show no significant alterations between the different storage conditions and remain at approximately 80% water content throughout the period.

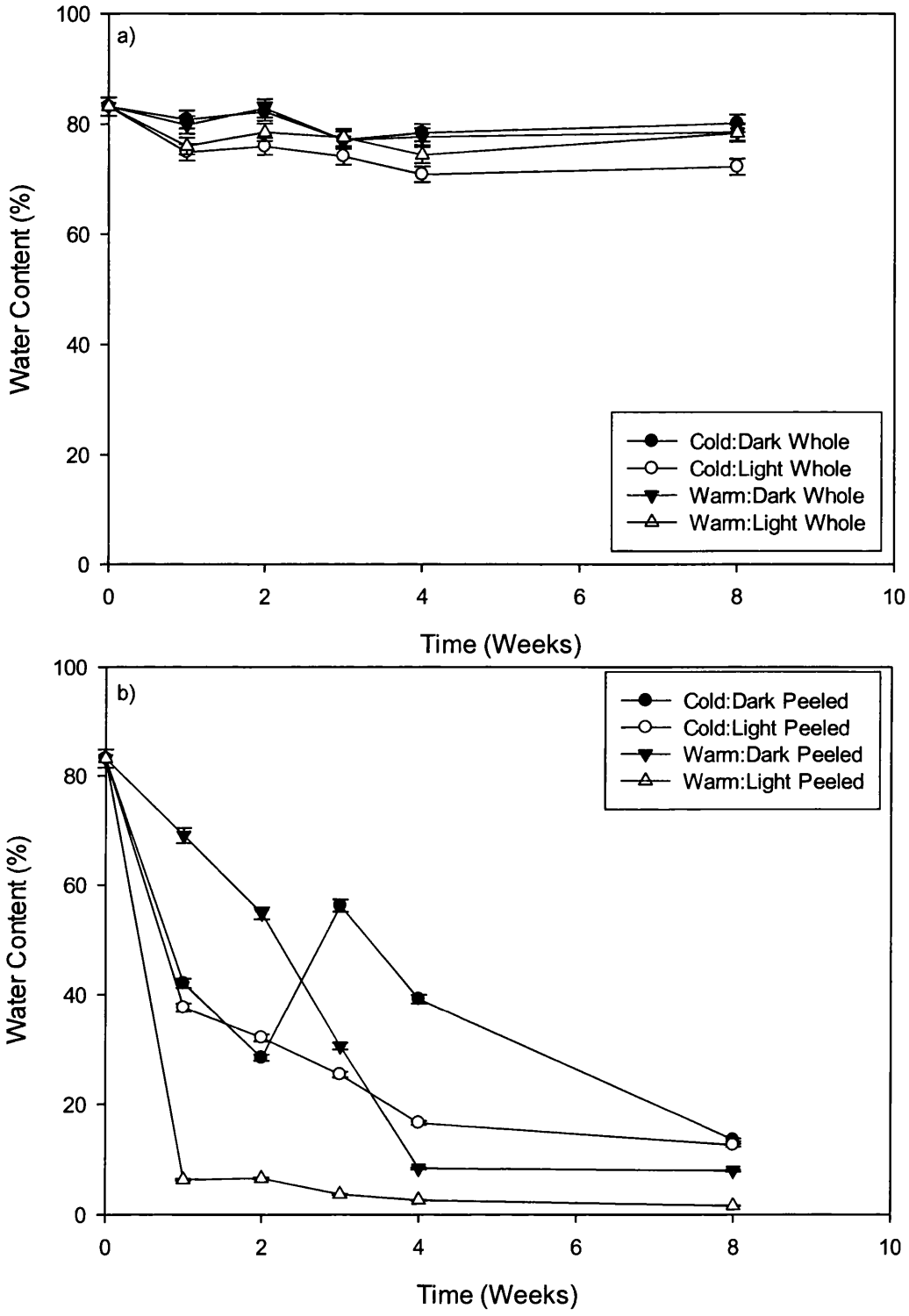

Figure 6.1.2: Water Content of the potatoes over the 8 week storage period, a) whole potatoes and b) peeled potatoes

These results suggest that tubers when stored whole with undamaged peel suffer minimal water loss; although the actual period of time studied is comparatively short compared to some long term storage studies of potatoes over non-growth periods (21 weeks – Cheong, 1998; 28 weeks - Hajirezaei et al. 2003; 12 weeks + 4 post harvest – Frančáková et al. 2011). The water content of the stored peel is shown in Figure 2b and varies significantly when compared with the whole tubers. The initial water content was 83%, the same as for whole tubers, and declined over the course of the 8 week period. However, there is a noteworthy difference between the storage conditions on the rate of peel dehydration. The peel stored in the warm and light conditions suffered a 76.8% loss of water in the first week alone compared with the peel stored in the warm and dark conditions losing only 14.1 %. This is believed to be due to the drying effect of being exposed to the warm and light conditions as opposed to the warm and dark conditions which was at a much higher humidity. The air transfer rate in the warm-dark box was low despite the holes, resulting in a build-up of humidity. Despite this, the warm and dark stored peel eventually reached a water content of 7.9 % by the end of the storage period. The lowest water content was found as expected in the peel stored in the warm and light conditions, with final water content at the end of the eight week period being 1.6 %. Peel stored in the cold conditions displayed similar water loss rates over the first two weeks, although strangely the peel stored in cold and dark conditions displayed an increase in water content at week three; thought to be due to increased humidity in the storage container. The humidity inside the container would increase due to potato respiration, and with a low air transfer rate the humidity would remain within the container, therefore reducing the driving force for dehydration to occur (Voss, 2014). A control of this variable was not undertaken, i.e. measurement of the humidity of an identical empty box, however the effect of potato respiration is the dominant cause of the increased humidity. The results suggest that the process of storing waste potato peel after processing of potatoes would see significant drying of the peel in a short time period. Potentially, the investigation of peel drying methods could be studied for the longer term storage of potato peel. However, this is dependent on the compositional changes with storage time making peel storage worthwhile.

Protein composition

The protein content of a whole potato is known to be approximately 1.5% of the total tuber on a fresh basis and approximately 8 % on a dry basis (Lister and Munro, 2000; Riley, 2010). The initial protein content of the potatoes studied was found to be 1.46 g/100g dry weight which equates to 1.46 %. This value is significantly lower than the 8% value reported in the literature; however, a value of 1.5 % has been previously reported by Bártová et al. (2013). The protein content remained almost constant throughout the eight week storage period displaying only very slight fluctuations. This is a similar result to the findings reported by Pinto et al. (1993) who studied potato storage over a period of 180 days finding a protein content of between 4-6 % on a dry basis. In this case, no remarkable differences could be noticed between the different storage conditions and both the peeled and whole potatoes exhibited a similar behaviour suggesting protein content remains constant over time.

Sugar analysis

Figure 6.1.3 shows the total sugar content of the whole potatoes and peel over the eight week storage period. The initial total sugar concentration was found to be 15.1 g/100g fresh weight (FW) which corresponds to values found in the literature reported to be between 12.6 and 18.2% for starch and soluble sugars between 0.01 and 0.68 % (Lister and Munro, 2000; Singh and Kaur, 2009). The total sugar level can be seen to generally decrease for the both the whole and peel potatoes with increasing storage time. In the case of the whole stored tubers, the total sugar level is seen to decrease from the initial 15.1 g/100g FW to approximately 10 g/100g FW in a one week period for the tubers stored at all conditions except the ones stored in the cold light conditions. The total sugar content in the whole potatoes continues to decrease, albeit at a slower rate than observed in week one, with the final sugar content after 8 weeks of storage at various conditions in the range 5.5 - 6.9 g/100g FW. Similarly to the whole potatoes, the total sugar concentration of the stored potato peel is seen to decrease from the starting value of 15.1 to approximately 9

g/100g FW for all conditions, although an exception is the peel stored in the warm dark conditions which remained at 13 g/100g FW after a one week period.

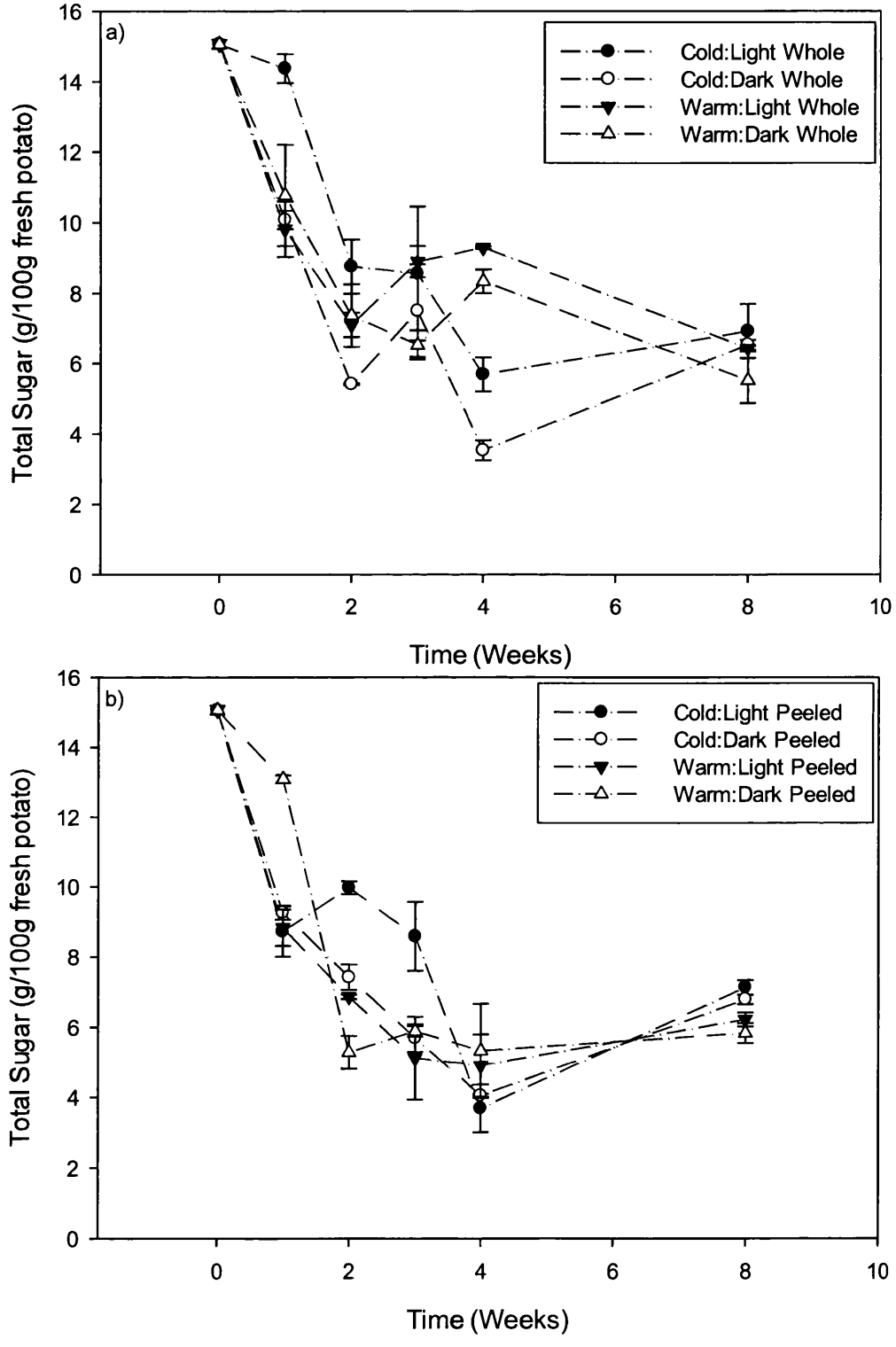

Figure 6.1.3: Total sugar content of the potatoes over the 8 week storage period, a) whole potatoes and b) peeled potatoes

The total sugar content in the stored peel continues to decrease over the 8-week storage period with the final sugar content at various conditions in the range 5.8 - 7.1 g/100g FW which is similar to the potatoes stored whole. In both cases the tubers that exhibited lowest total sugar were the ones stored in warm dark conditions whereas the tubers that exhibited the highest total sugar where the tubers stored in cold and light conditions. The decreasing total sugar concentration may be explained by considering the starch breakdown occurring throughout the storage period, where starch may be converted to reducing sugars (Voss, 2014). In the majority of studies conducted, the potato composition is often measured by analysis of the whole tuber (Hajirezaei et al., 2003; Frančáková et al., 2011), whereas this study only analysed the peel. Starch levels were found to decrease with storage in studies by Nielsen et al. (1997) and Hajirezaei et al. (2003) where the results found reducing sugar levels increasing, particularly under cold storage conditions. A similar pattern is observed in this work, although the levels of reducing sugars were not monitored in this case, and furthermore the ratio of soluble sugars to starch is low therefore would be masked by total starch content. Further to the conversion of starch to simpler sugars the overall quantity of sugars and starch decreases through the process of tuber respiration, where starches and sugars are converted to carbon dioxide (Butchbaker et al., 1973; Nourian et al., 2003).

Calystegine Analysis

The mass of calystegine in the different storage conditions in whole and peeled potatoes was studied for an eight week period and is shown in Figure 6.1.4. The initial concentration of calystegine B_2 was 44.8 mg/kg on a dry weight basis. This value is low when compared to other literature studies where values for dried peel are reported in the range 153 to 2100 mg/Kg on a dry basis (Friedman et al., 2003). However, Friedman et al. used a methanol water extraction method for 24 hours as opposed to the water only method used in this study. Figure 6.1.4 indicates varying levels of calystegine B_2 throughout the 8 week period in both the whole and peel stored potatoes. In the case of potatoes stored in warm and light conditions the calystegine level increased in the first week with levels increasing to 124 and 94

mg/kg for the whole and peel stored potatoes respectively. A similar pattern was observed in the potatoes stored in cold and dark conditions, however, the increase in stored peel concentration showed a much greater increase than that of the whole stored potato (101 and 65 mg/kg respectively). Despite the initial increase in calystegines levels, the potatoes stored in the cold dark conditions experienced a significant decrease in calystegine B_2 levels following week 1 with final levels of 13.4 and 0.2 mg/kg for the whole and peel stored tubers respectively at the end of the 8 week storage period. Overall the concentration of calystegine observed in the whole stored tubers is greater than those stored as peel. This may be due to the significantly changing water conditions over the eight week storage period studied in which the calystegine compounds are damaged by potato dehydration.

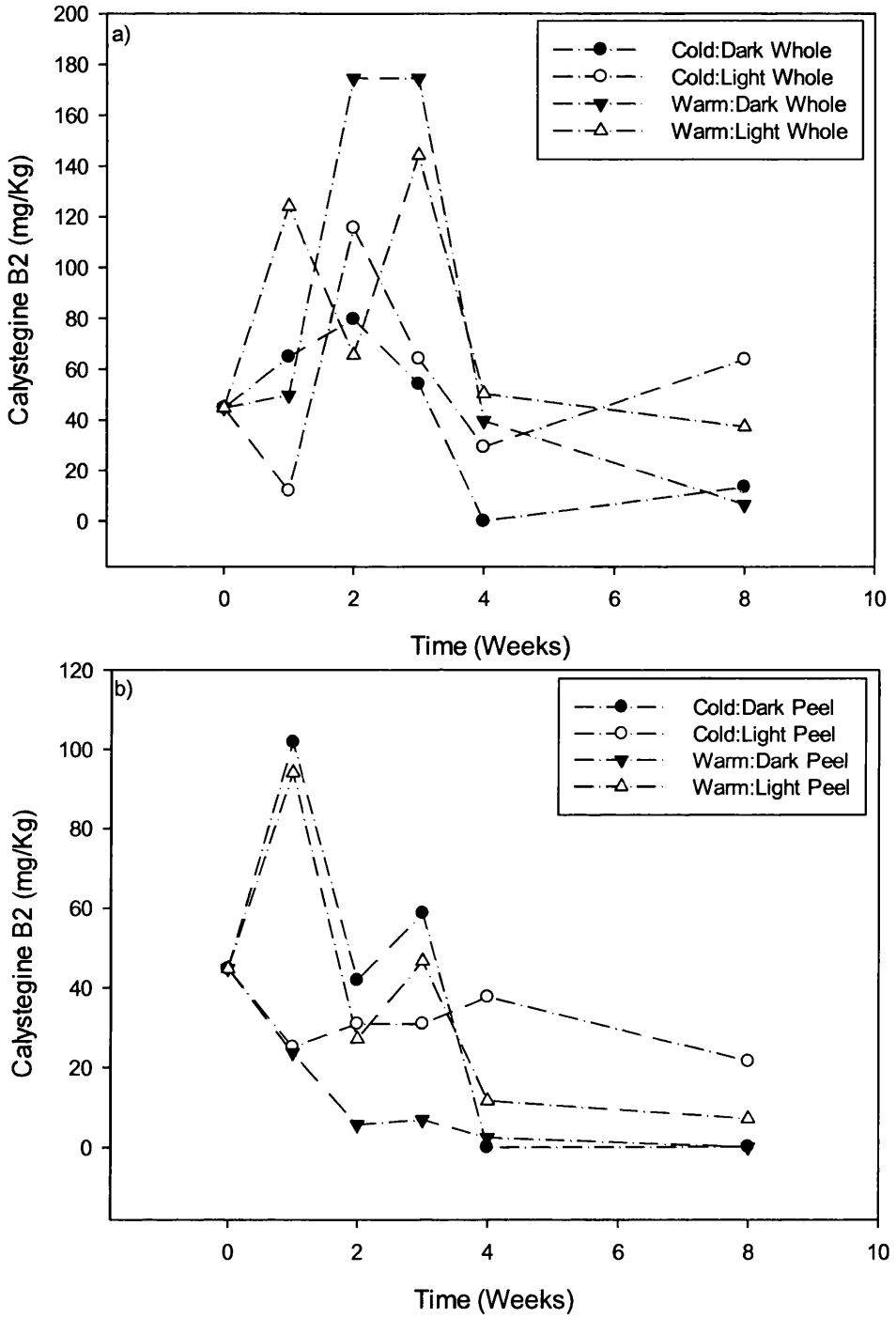

Figure 6.1.4: Calystegine B2 content of the potatoes over the 8 week storage period, a) whole potatoes and b) peeled potatoes (Analysis performed by GC)

Generally, the calystegine concentration is seen to decrease over the eight week storage period, although in both the whole and peel stored tubers the final concentration is seen to be higher in the potatoes stored in the cold and warm

conditions that were both exposed to light. The highest calystegine concentration in both cases was in the tubers that were stored in cold and light conditions where the concentration in the whole and peel stored potatoes were 63.8 and 21.5 mg/kg respectively. Overall, the final concentration was lower in the peel stored potatoes as opposed to the whole stored potatoes where the calystegine levels ranged from 0.1 – 21.5 mg/kg and 6.5 - 63.8 mg/kg respectively. Furthermore the calystegine levels were higher in each corresponding storage conditions for the potatoes stored whole at the end of the studied period when compared to the stored peel. The low levels of calystegine detected in this study may be attributed to the extraction method used. Traditionally studies have used organic solvents, in particular methanol for the extraction of calystegine from dried plant material (Richter et al., 2007; Griffiths et al., 2008), however the processing method developed is sensitive to such solvents therefore this study has avoided the use of organic solvents.

Extraction Performance

Sugar and Protein Extraction

The sugar and protein extraction rates are shown in Figure 6.1.5. Figure 6.1.5a and 6.1.5b show the sugar extraction rate over a 90 and 30 minute period respectively. Figure 5a shows that regardless of the size of potato skin (peeled or chopped) the extraction rate seems to decrease to zero after a period of about 30 minutes with the fastest extraction rate being seen in the first 10 minutes. In Figure 5b the time axis is shortened displaying the rate of extraction in the first stages of the experiment.

The effect of stirrer speed and peel size can be seen quite clearly on both figures 5a and 5b. In both cases (peel and chopped) a stirrer speed of 0rpm results in the slowest extraction rate as well as the lowest overall extraction quantity, reaching a maximum sugar extraction quantity of 31 and 35 mg/L after a period of 90 minutes for the peel and chopped respectively. However, this pattern is not repeated for all the stirrer speeds used for the experimentation. For instance, at a stirrer speed 180 rpm the peel reached a maximum sugar concentration of 106 mg/L whereas the chopped skin reached 261 mg/L.

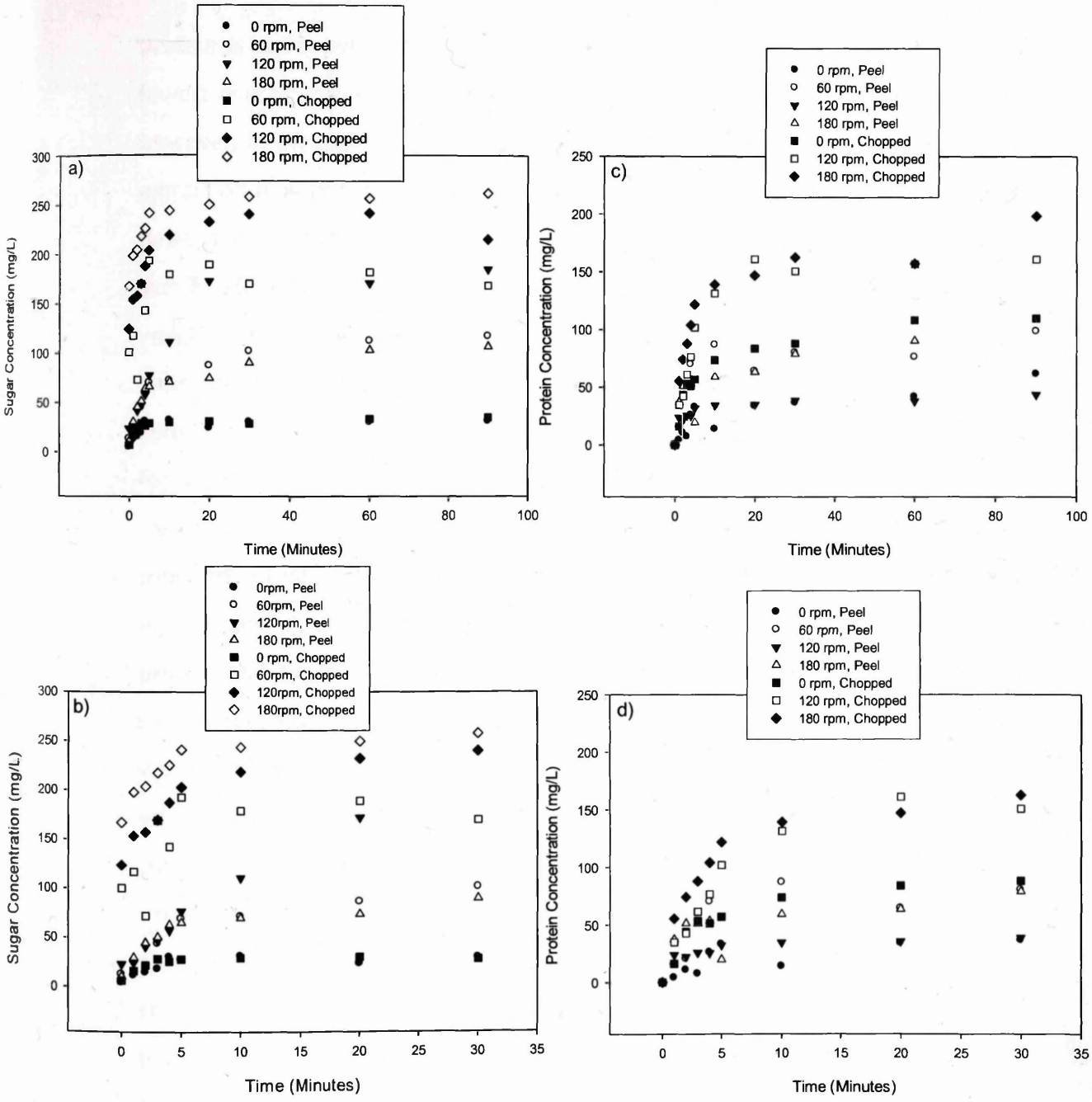

Figure 6.1.5: Sugar and Protein extraction rates at various conditions

In the experiments undertaken the chopped peel reached a higher overall concentration after 90 minutes in almost all cases, this suggests that the finer the peel the more efficient the extraction rate. The values obtained for the sugar profiles account only for the soluble sugars present in the potato, a value which is much lower than the results reported in Figure 6.1.3 where the total sugar (starch is insoluble in water) is also reported. In terms of protein extraction rate a similar relationship is seen as shown in Figure 6.1.5c and 6.1.5d, where the majority of the

protein is extracted within the first 20 minutes. The slowest extraction rate and lowest overall amount of protein extracted was observed with the peel at 0 rpm, however, in the case of protein extraction the chopped samples achieved a higher extraction quantity in all cases suggesting that the protein extraction rate is highly dependent on peel size. The highest extraction value was obtained in the chopped skin at 180 rpm where a concentration of 196 mg/L was obtained. 100g of fresh weight potatoes contains approximately 2g of protein, in this study the maximum concentration of 196 mg/L corresponds to approximately 10% of the total protein contained in a potato. However water is often not used as an extraction medium for protein hence the low extraction efficiency. The results obtained in the extraction study would suggest that the sugars and proteins being extracted are free and not intercellular components of the potato peel. The process of chopping up the peel would disrupt the cell wall and hence result in a greater quantity of protein and sugar to be extracted. The cell walls of the whole peel would have experienced much less damage and therefore a greater majority of the cell walls would be intact. The higher levels of sugar and proteins extracted when the peel was chopped further supports the hypothesis displaying the effects of a greater surface area. The effect of stirrer speed can be seen in both the sugar and protein extraction quantities in Figure 6.1.5. The results show that the higher the stirrer speed the higher the levels of sugar and protein extracted. This suggests that the stirrer speed promotes a higher extraction efficiency. This effect may be attributed to the stirring effect removing concentrated solution away from the sample surface allowing new solvent to come into contact with the solid surface or through the increased turbulence inside the extraction vessel allowing greater penetration of the solvent into the sample matrix. There is also a possibility that the shear caused by the increased stirrer speed may cause some level of cell disruption when the solid samples come into contact with the rotating impeller. The extraction quantity is limited by the quantity of proteins and sugars available outside of the potato cells. In order to further increased the quantities of protein and sugars extracted the cells would need further disruption through homogenisation or maceration.

Calystegine Extraction

The calystegine extraction rate is shown in Figure 6.1.6.

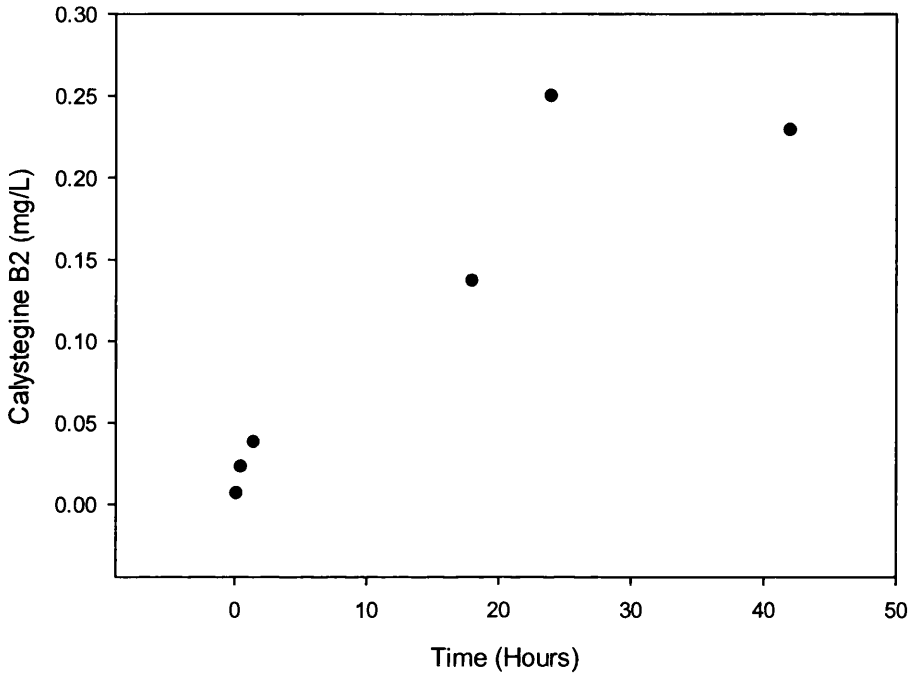

Figure 6.1.6: Calystegine B2 extraction rate at room temperature and 0 rpm

Initial scoping work undertaken suggested that calystegines are readily extracted using water alone, however, these results were variable and unreliable due to the low extraction rate and relatively low initial concentration in the potato. This initial work tested the same samples as those used for the sugar and protein extraction study and found that very small amounts of calystegine compounds were extracted from the potato material in the first few minutes. In contrast, samples obtained from an industrial supplier had very high levels of calystegines present in the water where the potatoes had been left for a few days, suggesting that water was suitable for the extraction albeit over an extended time period. In the initial study, the effect of peel size was unclear due to the lack of data regarding extraction rate. Many of the previous studies undertaken have reported using organic solvents as an extraction solvent with some reporting extraction times of 24 hours (Friedman et al., 2003), although some studies have reported extraction times as low as 30 minutes (Petersson et al., 2013). An extraction study into the rate at which

calystegine is released from plant material has not been previously undertaken to the best of the authors' knowledge. Figure 6.1.6 shows the extraction rate of calystegine B_2 from fresh plant material into water. The results obtained show that after a period of 1.5 hours, a calystegine weight of 0.038 mg/L of water has been extracted; this further increased a value 0.14 mg/L after a period of 18 hours before tapering off to approximately 0.23 mg/L of water after 42 hours. These values were observed at room temperature (20°C) with a 0 rpm stirrer speed. Furthermore the calystegine extraction study was undertaken at a large scale in order to minimise any error in using very small extraction volumes, where actual calystegines may be missed due to the extremely small quantities and the limits of resolution of the analysis method.

In terms of process design, the rate at which the sugar and protein components extract compared to the calystegine compounds would suggest that the introduction of a two stage extraction would be beneficial to the overall process efficiency. The protein and sugar extraction rate declines dramatically after a period of 30 minutes whereas the results obtained showed no detectable calystegines after the same time period. The results obtained would suggest that the calystegines are mainly intracellular compounds of the potato, with Keiner and Drager (2000) reporting that calystegines are mainly present in the young meristematic tissues of the potato. The extraction rate of the calystegines from within the undamaged cell would be through diffusion from within the cell membrane. In order to increase the extraction rate the potato peel could be macerated or homogenised in order to damage the cells further, however, this would result in a much greater level of sugar and protein extraction. This may be further studied by comparing the extraction rate and quantity with variations such as particle size (peeled, chopped, homogenised), temperature, pressure and solvent. The two-extraction method would be achieved through the application of a 30 minute water extraction, prior to flushing and re-extracting with fresh water should reduce the levels of sugar and protein present and therefore result in a purer calystegine product for downstream processing. The reported 1 to 10 mg/kg fresh weight of calystegines present in potatoes (Andersson, 2002) combined with

the approximately 300 million tonnes of potatoes grown per year suggests that should a feasible extraction process be designed the large scale extraction of calystegines from potatoes is viable. Considering that not all of the mass of potatoes grown would be suitable for calystegine extraction, even a small percentage of this total would provide a significant supply of calystegine compounds.

6.1.4 Conclusion

This study has investigated the effect of storage time and conditions on the composition of potatoes with the aim of maximising calystegine content for recovery. The study investigated storing whole potatoes as well as potato peel which is similar to what would be obtained from an industrial food supplier. The study undertaken aimed to replicate industrial storage conditions. Furthermore the effect of storage time on potato composition was investigated as the study aimed to determine the maximum calystegine level prior to recovery.

The results obtained showed water content of a whole potato remains almost unchanged over an eight week storage period, however, potato peel observed a significant and rapid moisture loss over the same period. The protein content of the whole potatoes and potato peel was found to remain constant throughout the storage period. The total sugar content of the tubers in both whole and peel form decreased over the period studied to almost similar levels of 6 g/100g fresh weight of potato. This is due to a combination of starch degradation, where starch is converted to simpler sugars and through potato respiration, where starch and sugars are converted to carbon dioxide in the presence of oxygen. Calystegine levels (in particular levels of calystegine B_2) were seen to decrease over the storage period in both whole and peel stored potatoes. However, the levels of calystegine B_2 in the peel was lower than that of the whole tubers suggesting the dramatic loss in moisture over the period studied may have had an effect.

Further to the composition study, the extraction of soluble sugars, protein and calystegine B_2 was also studied. The results obtained showed the clear effect of the stirrer speed with the lowest extraction rate being observed with a speed of 0 rpm

in both the peeled and chopped samples. The highest sugar levels were observed with a stirrer speed of 180 rpm with the chopped samples reaching a concentration of 261 mg/L whereas the peeled samples reached a maximum concentration of 106 mg/L. The significant difference between the chopped and peeled samples may be attributed to the extra damage caused to the cell walls during preparation which results in large quantity of sugars being available for extraction. Similar results were obtained for the extraction of proteins, with the chopped peel reaching a higher concentration at all stirrer speeds when chopped compared to the peeled samples. The highest concentration of protein was obtained with chopped peel at 180 rpm where a concentration of 196 mg/L was achieved. This study has shown that the majority of the soluble sugars and proteins are extracted into water within the first ten minutes of the extraction process. The calystegine is released much more slowly into the water reaching a maximum in a period of 24-48 hours. This would suggest that rinsing the potato peel with a volume of water following initial extraction and prior to calystegine extraction may help reduce the quantity of sugars and protein present and simplify further processing.

Overall this study suggests that large scale extraction and purification of calystegines should be undertaken using freshly peeled potato skin, chopped into small pieces and extracted over a period of 48 hours into water using agitation. This process should provide the optimum conditions in order to maximise product recovery.

6.2: Membrane Technology for Industrial Scale Calystegine Recovery

6.2.1 Introduction

This section discusses the feasibility and development of a membrane process suitable for the large scale extraction and purification of calystegines from potato peel waste. The work investigates the use of laboratory based microfiltration and nanofiltration equipment. The findings of the laboratory scale study were then implemented on a pilot scale using again both microfiltration and nanofiltration systems representative of a full scale processing system. The overall aim of this work was to study the feasibility of membrane technology for the separation and purification of calystegines from a low value waste product, and ultimately produce a potato extract containing high levels of calystegines. Production of such an extract would allow calystegines to be further studied for their beneficial properties, either as an extract of through further purification using traditional ion exchange methods.

6.2.2 Materials & Methods

The same potato stocks were used for the laboratory based membrane study as per the extraction study in Section 6.1.

For the laboratory based studies, 500 g of fresh potato peel was added to 5 litres of ultrapure water. Hand peeled strips were added to the water at 20 ± 2 °C and stirred at 60 rpm for 24 hours to ensure maximum extraction. The extraction time was shown sufficient from the previous extraction study. The 10:01 ratio of water to peel (weight basis) was maintained throughout all the experimental work. The 10:01 ratio was selected in order to produce an as water like process fluid as possible, and to ensure that there was an excess of extraction fluid available. The reasoning behind selecting a 10:01 water to peel ratio was in order to ensure the extraction solvent was in excess to ensure maximum extraction.

The potato peel and potato water for the pilot scale studies was kindly donated by 2 Sisters Food Group (Rogerstone, Gwent, UK). The peel and water samples donated were obtained directly from the food manufacturing process and therefore

were representative of an industrial potato waste. The feed material was produced, collected and processed within 72 hours to help maintain freshness and prevent spoilage of feed material.

For the pilot scale studies, 20 kg of the industrially obtained potato peel was added to 200 L of clean tap water. The extraction was undertaken at 20 ± 2 °C and was periodically stirred to ensure maximum extraction over a period of 48 hours. The industrial potato water was processed separately

The laboratory based microfiltration studies were undertaken in an Amicon 8050 frontal filtration cell using the membranes as described in chapter 3. The laboratory scale nanofiltration study was undertaken in the Membranology cell with the using the membranes as described in chapter 3. The feed used for the nanofiltration process was the permeate of the optimum microfiltration membrane pore size.

The pilot scale studies were undertaken in the pilot scale equipment as described in Chapter 3. A 0.2 µm membrane (CFP-2-G-55, GE Healthcare Life Sciences, Little Chalfont, UK) was used for the pilot scale microfiltration while a NTR-7450 membrane was used for the pilot scale nanofiltrations.

All analysis was carried out as described in Chapter 3, with the calystegines analysed as described in Chapter 6.

6.2.3 Results and Discussion

6.2.3.1 Laboratory Scale Study

Process development

The calystegine compounds (B2 – MW 175, A3 - MW 159) of interest have molecular weight within the nano-scale range and therefore there is potential for separation and purification by nanofiltration (< 1000 MW). The initial process scoping realised that the calystegines of interest are a very small percentage of the total potato mass, therefore in order to produce a feasible process a large quantity of feedstock would be required to generate enough product mass. Therefore, any process that is to be implemented is required to process large quantities of feed

stock while attempting to simultaneously perform a high resolution separation. The varied nature of the feed would require the designed process to operate over a wide variety of feed conditions. The nature of the potato peel obtained from industrial processing will vary significantly dependent on potato variety used, time of year (potato age), processing (peeling) method and peel storage time (after peeling). The designed process would be required to be as cost effective as possible, the development of a labour intensive and costly process would lead to an unfeasible process. Furthermore, the process designed would either be required to be 'skid' mounted in order to add on to existing potato processing facilities or a larger centrally located processing facility where the waste potato peel is collected and processed together.

Microfiltration (MF)

The initial study researched the implementation and optimisation of a prefiltration stage in order to clean up the potato waste stream prior to nanofiltration. The nature of the potato extraction both at laboratory and industrial scales results in small particulates being present in the process feed. The quantity of soil debris and potato particulates (loose skin, plant matter) are minimised in the laboratory scale experimentation due to the potatoes being shop bought, cleaned and hand peeled, however, feed obtained from industrial processing plants are likely to contain higher levels of debris and particulates.

Microfiltration technology has developed greatly in the last 40 years and now finds many uses in dairy and food sectors (Daufin et al., 2001) often used for clarification of fruit and vegetable juices. The use of microfiltration to process the initial extraction feed stock would allow the production of a standardised feed for downstream processing through the effective removal of particulates present. Furthermore, the potato extraction stage releases a large quantity of starch from the potato peel into the extraction water, which would affect the processing efficiency of downstream operations through heavy fouling of the membranes. Interestingly, starch is only slightly soluble in water at 20°C therefore the starch will often form a insoluble 'scum' layer that would essentially act like a glue fouling any

membrane process to which the feed is introduced. Therefore a process which can remove all particulates and reduce any starch present is essential to the overall process feasibility.

The results of the initial MF study are shown in Table 6.1.2. The membranes used were all cellulose acetate supplied by Millipore UK Ltd. (Watford, UK). The pore sizes quoted are nominal pore sizes as quoted by the manufacturer. As these membranes are microfiltration membranes the pore resolution is high and therefore the pore size distribution will be low.

Table 6.2.1: Mean permeate particle size and flux rates of laboratory scale microfiltration study

Nominal Membrane Pore Size	Pressure	Mean Particle size	Membrane Permeate Average	Standard Deviation	Flux
(um)	(bar)	(Z_{ave}, nm)	(nm)	(nm)	(LMH)
5.0	0.1	113.3	110.3	9.5	121.5
	0.3	104.9			-
	0.5	100.9			-
	1	122.2			-
1.2	0.1	46.6	65.8	15.9	66.5
	0.3	79.6			69.5
	0.5	58.9			-
	1	78.0			-
0.8	0.1	26.0	25.6	3.2	38.2
	0.3	24.3			45.9
	0.5	19.7			57.1
	1	32.4			-
0.2	0.1	10.9	10.6	1.5	42.4
	0.3	9.6			44.5
	0.5	9.4			72.8
	1	12.7			84.1
0.05	0.5	0.8	3.6	4.0	20.0
	1	6.5			20.4

Five microfiltration membranes were selected for the study and the results obtained showed significant trend between membrane pore size and mean permeate particle size, with particle size decreasing as membrane pore size decreased. The mean permeate particles size for the 5 μm membrane ranged from 100.9 nm to 122.2 nm with no observable pattern between membrane operating

pressure and mean permeate particle size. In fact, no pattern between particle size, membrane pore size and operating pressure is observed over the range studied. The average particle size of the microfiltration membranes permeates is shown in Figure 6.2.1 where the distinct relationship between membrane pore size and permeate particle size is clearly observed.

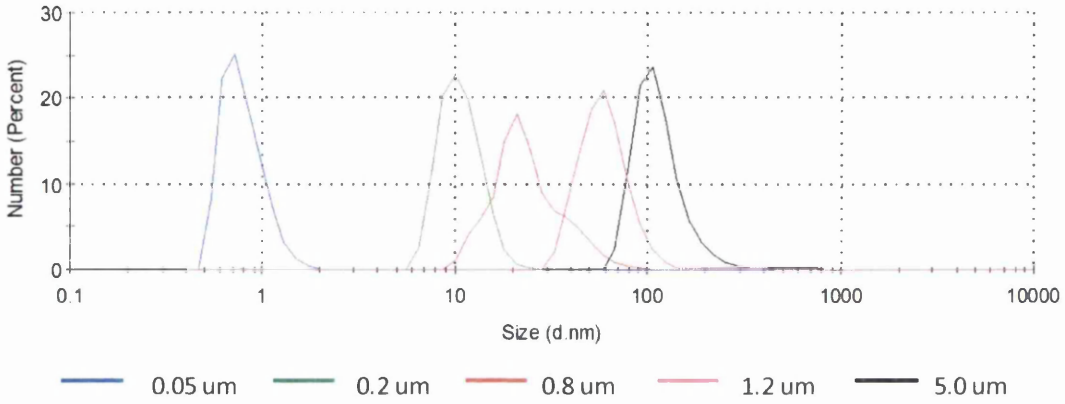

Figure 6.2.1: Mean particle size of microfiltration permeate

The largest permeate particle size is seen with the 5 µm membrane where a average particle size of 110.3 nm was observed, with the 1.2 and 0.8 µm membranes producing a mean permeate particle size of 65.8 and 25.6 nm respectively. The mean permeate particle size produced by the 0.2 and 0.05 µm was 10.6 and 3.6 nm respectively. The initial results suggest the use of the 0.05 µm membrane, due to the production of the smallest average permeate particle size, however, the flux rate obtained with the 0.05 µm was quite low (shown in Table 1). The highest flux rates were obtained using the 5 µm membrane as expected, although the flux was so large at 0.3, 0.5 and 1 bar that the permeate flow exceeded the measurement limitations of the equipment. The same observation was made using the 1.2 µm membrane at 1.5 and 1 bar and the 0.8 µm at 1 bar. The lowest flux was observed with the 0.05 µm membrane where a flux rate of 20.0 and 20.4 LMH was obtained at 0.5 and 1 bar respectively suggesting that the membrane experiences fouling. The flux rates obtained using the 0.2 µm membrane were higher than those obtained using the 0.05 µm membrane and furthermore the 0.2 µm membrane exhibited less fouling (flux decline). The 0.2 µm membrane was successful removing the particulates present and therefore will be

selected as the prefiltration membrane pore size despite the larger average permeate particle size than that of the 0.05 µm membrane as the flux rates obtained were higher.

Nanofiltration

The initial scoping work showed the presence of a large amount of sugars and amino acids in the nanofiltration feed. This resulted in the selection of the membrane being of utmost importance with the main aim being to remove as much of the interfering compounds as possible to essentially leave as pure a calystegine extract as possible. Thus, four nanofiltration membranes were selected, ranging from a MWCO of 1000 to approximately 150-300. Similarly to all nanofiltration operations a high flux along with a high rejection is preferable. Table 6.2.2 displays the flux performance of the four membranes studied during the nanofiltration of the pre-filtered potato extract. The initial clean water flux measurements were taken post pre-compaction and before any potato extract was processed. The results obtained suggest that the clean water flux for each membrane studied is similar with an average clean water flux of 7.54 LMH/bar over the Desal HL, NF 270 and NTR-7450 membranes, however, the average clean pure water flux observed with the UF GE membrane was 1.56 LMH/bar. This value is surprising as the UF GE membrane has the largest reported MWCO of 1000. The average pure water flux for the Desal HL, NF 270 and NTR-7450 are very similar with values of 7.96, 7.28 and 7.39 LMH/bar respectively. A larger membrane MWCO would normally suggest a more open pore structure and therefore a higher flux rate, however this is not reflected in the experimental findings. All the nanofiltration membranes studied in this work were thin film composite membranes. The thickness of the active layer plays a vital role in the flux rates achievable by a nanofiltration membrane. A thicker active layer produces much greater resistance to flow and therefore reduces the flux rates. The UF GE membrane has an active layer thickness of 29 µm (Adnan et al. 2012), while the NTR-7450, NF 270 have active layer thickness of 11.69 µm (Bargeman et al., 2005), and 15-40 nm (Simon et al., 2013) respectively (Thickness of Desal HL active layer unknown. The values reported in the literature show that the UF GE membrane has

the thickest active layer and would therefore have a larger resistance to flux when compared to the other membranes studied which have a thinner active layer.

Table 6.2.2: Potato extraction flux rates over the range of membranes studied

		Clean	Dirty	Flux	Sample Processing Flux		100 mL	Average Flux
		PWF	PWF	Lost			Processing Time	(100 mL)
	Pressure (bar)	LMH/Bar	LMH/Bar	%	LMH	% CWF	(Minutes)	(LMH)
Desal HL	5	7.63	7.29	4.39	27.10	71.07	57.50	24.80
	10	7.87	7.32	6.99	63.30	80.41	22.00	62.50
	15	7.95	7.24	8.90	89.30	74.88	15.25	90.80
	20	8.39	7.19	14.20	116.20	69.29	12.25	113.60
	30	7.98	7.53	5.59	173.50	72.48	5.25	163.00
NF 270	5	5.89	5.51	6.58	21.60	73.29	66.00	21.60
	10	7.69	6.86	10.73	54.30	70.64	25.25	56.00
	15	7.92	7.39	6.68	94.20	79.30	14.00	97.60
	20	7.76	7.23	6.91	118.20	76.14	11.50	119.60
	30	7.14	7.13	0.20	160.00	74.68	8.25	163.50
NTR 7450	5	6.22	2.68	56.85	13.30	42.79	92.50	15.40
	10	7.84	2.46	68.64	29.20	37.24	39.50	36.10
	15	8.16	2.86	64.96	43.90	35.85	27.75	51.00
	20	6.88	3.17	53.92	59.50	43.26	20.25	68.80
	30	7.86	3.76	52.13	96.80	41.06	13.50	105.20
UF GE	5	1.64	1.15	29.76	5.60	68.17	284.25	5.10
	10	1.56	1.24	20.21	11.60	74.41	120.25	12.20
	20	1.56	1.30	16.88	23.20	74.45	60.50	23.50
	30	1.51	1.24	18.10	31.80	70.01	42.50	32.90

In each case 100 mL of sample was processed by each membrane studied. The highest flux was obtained by the Desal HL membrane where a flux of 173.5 LMH was obtained at 30 bar which is a flux value 72.5 % of the original clean water flux.

In trend with the clean pure water flux rates the UF GE observed the lowest sample processing flux rate with a value of 31.8 LMH at 30 bar, which despite being 70.0% of the membranes clean water flux is only 18.3% of the flux observed with the Desal HL membrane. A similarly high flux was obtained when using the NF 270 membrane where a flux of 160.0 LMH at 30 bar was observed. The NTR 7450 obtained a flux of 96.8 LMH at 30 bar, a value which may be attributed to the significant flux decline observed when using the membrane over the processing period. Such a significant flux decline was not observed with the Desal HL and NF 270 membranes, this is believed to be due to the smaller pore size associated with the Desal HL and NF 270 membranes which have smaller reported MWCO (150-300 for both) compared to a reported MWCO of approximately 800 for the NTR-7450 membrane. The interaction between membrane pore size and solute size is a key parameter when considering membrane fouling. A fouling material of slightly larger dimensions to the size of the nanopore may deposit within the entrance of the pore and thus partially block the pore and therefore reduce the flux rate, this is known as intermediate fouling. Should the foulant be of similar dimensions to the pore the foulant may become trapped within the pore and essentially produce a pore constriction effect where the size of the pore becomes reduced and therefore again reduces the flux observed, this phenomena is known as standard blocking. In a case where the dimensions of the foulant are much larger than that of the pore the material is unable to enter the pore and therefore does not constrict the flow through the pore unless significant surface fouling is observed, which would be known as complete blocking. In the case of the experiments undertaken the smaller MWCO membranes which would have the smallest pore size experienced less of a flux decline which therefore suggests that the fouling material present is larger than that of the membrane pores and thus did not block the membranes pores as readily. The comparison of the sample flux rates to the clean pure water flux allows the appreciation of the fouling the membrane observes. The Desal HL, NF 270, and UF GE obtained a sample processing flux that was on average 73.6, 74.8 and 71.8 % of the original clean water flux whereas the NTR-7450 membrane sample processing flux was 40.0 % of the original clean water flux, reaffirming that the membrane is experiencing fouling.

The membranes studied were rinsed, refilled with pure water and pressurised to determine the flux decline observed through the processing stage. The results obtained were varied across the four membranes studied, although overall the Desal HL and NF 270 displayed the lowest flux decline with an average decline of 8.0 and 6.2 % respectively. Interestingly the NF 270 membrane displayed the smallest flux decline of all the membranes studied with a flux decline of 0.2 % at 30 bar. The UF GE displayed a slightly larger flux decline of 21.2 % when compared to the Desal HL and NF 270 membranes, however the largest flux decline as observed during the sample processing fluxes was observed with the NTR-7450 membrane where an average decline of 59.3 % was observed. The data obtained displayed no relationship between flux decline and operating pressure over all four of the membranes studied.

The TOC rejections of the laboratory nanofiltration study are shown in figure 6.2.3.

Table 6.2.3: TOC rejection in laboratory scale nanofiltration

	TOC Rejection (%)			
Pressure (bar)	**UF GE**	**NTR-7450**	**Desal HL**	**NF 270**
5	-	52.1	64.6	18.9
10	63.7	54.6	59.1	39.6
15	49.8	62.1	51.3	46.0
20	61.4	62.2	49.1	48.0
30	52.1	55.5	52.4	51.6

Over the pressure range studied, the average TOC rejections were 56.8, 57.3, 55.3 and 40.8 % for the UF GE, NTR-7450, Desal HL and NF 270 respectively. The TOC rejection values obtained for the nanofiltration membranes are surprising as the TOC rejections are similar despite the reported differences in membrane MWCO. The lowest TOC rejection being observed with the NF 270 is highly surprising as along with the Desal HL the membrane has the lowest MWCO. Overall the average TOC rejection across the four membranes studied was 52 % suggesting 48% of the

total organic carbon would be found in the permeate. The presence of such a large quantity in the permeates suggest compounds such as simple sugars and amino acids are permeating the membranes, however as is the case with all nanofiltration separation the rejection value is dependent on feed concentration, therefore the presence of a large quantity of simple sugars and amino acids would result in lower than expected membrane rejection. The results obtained would suggest that the membrane separation is not governed by size exclusion alone and that a charge exclusion mechanism is partially responsible. The low TOC rejection values obtained would suggest that the majority of charged species such as the amino acids are not being excluded on size and hence are permeated by the membrane. The NF stage could be further optimised by investigating the effect feed pH (i.e. membrane charge) has on the rejection. The results show that membrane operating pressures have no effect on the TOC rejection values.

In order to further investigate the membrane rejection properties the sugar and protein rejection of the nanofiltration membranes was studied and the results are shown in Table 6.2.4.

Table 6.2.4: Sugar and protein rejections using nanofiltration membranes

	Sugar Rejection (%)				**Protein Rejection (%)**			
Pressure (bar)	**UF GE**	**NTR-7450**	**Desal HL**	**NF 270**	**UF GE**	**NTR-7450**	**Desal HL**	**NF 270**
5	69.6	84.2	93.6	97.2	56.5	84.2	94.5	93.2
10	85.2	89.6	94.5	97.6	20.0	89.6	94.1	95.1
15	-	85.3	96.4	98.6	-	85.3	94.7	93.0
20	91.2	89.8	95.5	97.6	17.7	89.8	96.2	97.2
30	97.1	92.2	94.1	98.4	55.7	92.2	94.7	98.2

The UF GE displayed the lowest sugar rejection with an average sugar rejection of 85.8% over the range of pressures studied. The UF GE displayed the lowest overall

rejection with a value of 69.6% at 5 bar, however displayed a rejection of 97.1% at 30 bar. The average sugar rejection being lowest for the UF GE membrane is expected due to the membrane having the largest reported MWCO, although despite the largest MWCO a rejection of 85.8% is high. The NTR-7450 displayed an average rejection of 88.2% over the range of pressures studied, with the lowest rejection value of 84.2% obtained at a pressure of 5 bar and the highest rejection of 92.2% obtained at 30 bar. The Desal HL and NF 270 membranes both displayed the highest sugar rejection values with average rejections of 94.8 and 97.9% respectively. This is expected as the membranes are tight NF membranes and have the lowest reported MWCO values. Generally, the observed rejection increases with increasing operating pressure, although in the case of the Desal HL and NF 270 it would seem that the rejection profile is fully formed at 5 bar with no significant change in rejection across the whole range of pressures studied. The sugar rejection values obtained across the membranes studied suggests that size exclusion is the dominant mechanism. This is expected as sugars are uncharged particles and therefore would be unaffected by membrane charge effects. The results obtained displayed the lowest sugar rejection with the largest MWCO membrane, the highest rejections were obtained with the membranes with the lowest MWCO.

The protein rejection values obtained for the NF membranes studied are more variable than the observed sugar rejections. In the case of the UF GE membrane the average protein rejection was 37.5% which was the lowest average rejection of the four NF membranes studied. The range of values obtained for the UF GE membrane is interesting as rejection values of 56.5 and 55.7% are obtained at 5 and 30 bar respectively whereas rejection values of 20.0 and 17.7% are obtained at 10 and 20 bar respectively. The range of values is surprising, opposing the trend of increasing rejection with increasing pressure often seen with nanofiltration. An average protein rejection of 88.2% was observed with the NTR-7450 membrane across the range of pressures studied, the lowest rejection value of 84.2% was observed at 5 bar rising to 92.2% at 30 bar. Similarly to the sugar rejection results the Desal HL and NF 270 both displayed fairly similar results, with the NF 270 again displaying the overall highest average rejection value of 95.3%. The protein

rejections observed, similarly to the sugar rejections suggest that size exclusion is the dominant separation mechanism. However, it is possible that part of the observed rejection is governed by charge exclusion as some proteins and amino acids are charged. This exclusion mechanism may be further investigated through experimentation with feed pH in order to examine the effect membrane charge has on protein rejection. The Desal HL membrane displayed an average protein rejection of 94.8% with very little change in the rejection value across the whole range of pressures studied. The NF 270 displayed an increase in rejection over the pressure range from 93.2% at 5 bar to 98.2% at 30 bar.

The performance of the membranes for the permeation of the calystegine B_2 was studied in order to ascertain which membrane would be most suitable for the pilot scale trial. The Desal HL displayed total rejection of the calystegine while the NF 270 membrane allowed the permeation of a very small quantity of the compound (a permeate average of 0.02 ug/ml over the pressures studied). The almost total rejection of the calystegine suggests that the Desal HL and NF 270 membranes are suitable for the concentration of the calystegine compounds. The UF GE and NTR-7450 membranes did allow permeation of the calystegine compound with average concentration values 0.26 and 0.25 ug/ml respectively over the pressure range studied. The membranes due to their larger pore size allow for the permeation of the calystegine that is retained by the tighter nanofiltration membranes. The theoretical rejection of calystegine B_2 for the NTR-7450 membrane (MWCO - 400Da) is 57 and 71% at 10 and 20 bar respectively. Similarly to the work undertaken in Chapter 5 this values does not account for concentration polarisation, fouling, interactions with other species and pore size distribution. However, this would suggest that the NTR-7450 membrane would allow a quantity of calystegine B_2 to be permeated.

Membrane selection

Overall the laboratory scale experimentation has raised a number of interesting results. The selection of the membranes based on the results obtained would suggest that a 0.2μm microfiltration step followed by a nanofiltration process using

the NTR-7450 membrane. From the studies undertaken thus far the belief is that a 0.2 µm pore size is most suitable for the separation due to the efficient size reduction of the mean permeate particle size whilst maintain a steady processing flux. The membrane when compared to the 0.05µm produced a slightly larger particle size but exhibited a larger processing flux and a lower flux decline over the processing period. The nanofiltration study undertaken has suggested that the NTR-7450 membrane is optimum for the purification of the calystegine compound, however the results are not clear cut. The NTR-7450 membrane is selected mainly due to the high rejection of sugars and protein and whilst still allowing the permeation of some calystegine. The Desal HL and NF270 membranes despite their superior operating fluxes and low susceptibility to fouling resulted in almost total rejection of the calystegine along with very high rejection of sugars and proteins and therefore are not suitable for the purification of the calystegine. This would result in a concentrated calystegine fraction which would be very high in sugars and proteins and all compounds below 0.2 µm, therefore reducing the product purity. The UF GE membrane despite rejection a reasonable quantity of sugars and proteins and allowing the permeation of the calystegine displayed very poor flux performance (approximately 3x lower than NTR-7450) and therefore was deemed in terms of separation performance inferior to the NTR-7450 membrane.

6.2.3.2 Pilot Scale Study

Process Overview

In order to assess the feasibility of extracting calystegines on an industrial scale experimentation with pilot scale feeds and equipment is imperative. In this case a mock processing facility was designed and setup based on the results of the laboratory scale studies. The overall process designed attempted to implement a process that initially extracted the maximum quantity of calystegines into the aqueous phase prior processing by membrane technology, paying particular attention to reducing the presence of sugars and other contaminants in the final product. The initial feasibility work undertaken avoided the use of organic solvents (methanol) for extraction due to the potential future use of the product in the food

industry, the associated environmental impact and the inherent cost and safety aspects of using a large quantities of solvents. Overall the process development and experimentation on a pilot scale will allow the true feasibility of extracting calystegine compounds using membrane technology to be assessed and whether there is a future to the process.

The final process developed for the pilot scale feasibility study is outlined in Figure 6.2.2.

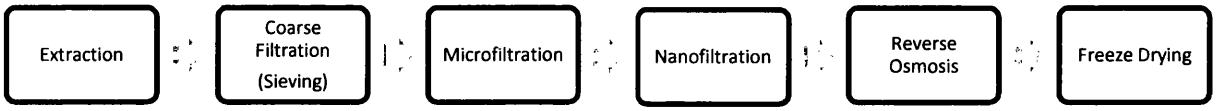

Figure 6.2.2: PFD of pilot scale calystegine extraction and purification process

The process begins with the extraction as previously mentioned, followed by a coarse filtration (sieving) stage where any particulate matter is removed prior to entering the microfiltration system. In reality the sieving stage may be combined with the microfiltration stage through the use of an in-line filter. The sieved feed enters the microfiltration unit and the process permeate was collected in clean food grade plastic barrels. These barrels then act as the nanofiltration feed where again the product is collected in clean plastic barrels. The final stage is the use of a reverse osmosis prior to freeze drying in order to reduce the volume of final product and speed up the drying process. In the case of the feasibility study each process was run as a separate unit operation, however should the process prove feasible, the operation may possibly be combined with a semi-continuous process with each unit operation feeding into the next.

The waste potato peel obtained from the industrial supplier is vastly different to the potato peel produced through the peeling of shop bought potatoes. Potato peel produced from peeling potatoes leads to a fairly clear extraction as shown in Figure 6.2.3. The industrial potato peel is very different to the large peeled strips obtained through manually peeling. The feed obtained from the industrial process plant

consisted of much smaller peel solids and formed a mulch. The industrial peeling process released a large quantity of starch from the potatoes that otherwise would be insoluble in water as can be seen by the scum layer in Figure 6.2.3. Overall, the industrial feedstock is vastly different to what can be replicated at a laboratory scale, a finding which would suggest that the experimentation undertaken at laboratory scale in this work is meaningless. Despite this the use of industrial feeds for fundamental studies is impossible due to the variability from batch to batch and as such, model solutions should be used. Model solutions provide a consistent and controllable basis that allow for isolation of specific process variables that may not be isolated using industrial feeds.

Figure 6.2.3: Laboratory (Left) and Pilot (Right) scale potato extractions

The membranes selected for the reasons mentioned above were a 0.2 µm microfiltration membrane in order to remove particulates followed by a NTR-7450 nanofiltration membrane in order to remove as much contaminants (sugars) from the final product.

Microfiltration

The pure water and processing fluxes for the pilot MF process are shown in Figure 6.2.4 and Table 6.2.5. The initial pure water flux obtained an average flux value of 495 LMH/bar prior to any processing. The pure water used throughout the

experimentation was freshly drawn tap water in all cases. The final pure water flux value obtained after all samples were processes was 112 LMH/bar a value which is 22.6 % of the original flux. The loss in flux of nearly 80 % over a relatively short period is of slight concern, suggesting that there is significant membrane fouling occurring during the processing. Further investigation of each processing stage saw almost a 50% decrease in pure water flux after run 1, suggesting the membrane experienced significant fouling at the start of the process (shown in Table 6.2.5). The subsequent steps exhibited a percentage clean water flux of 30.6, 27.1 and 22.5 % for the 1.0, 0.5 and 1.5 bar operating pressures (runs 2, 3 and 4) respectively. The values quoted were measured after a cleaning stage suggesting that the fouling which is believed to be starch is permanent.

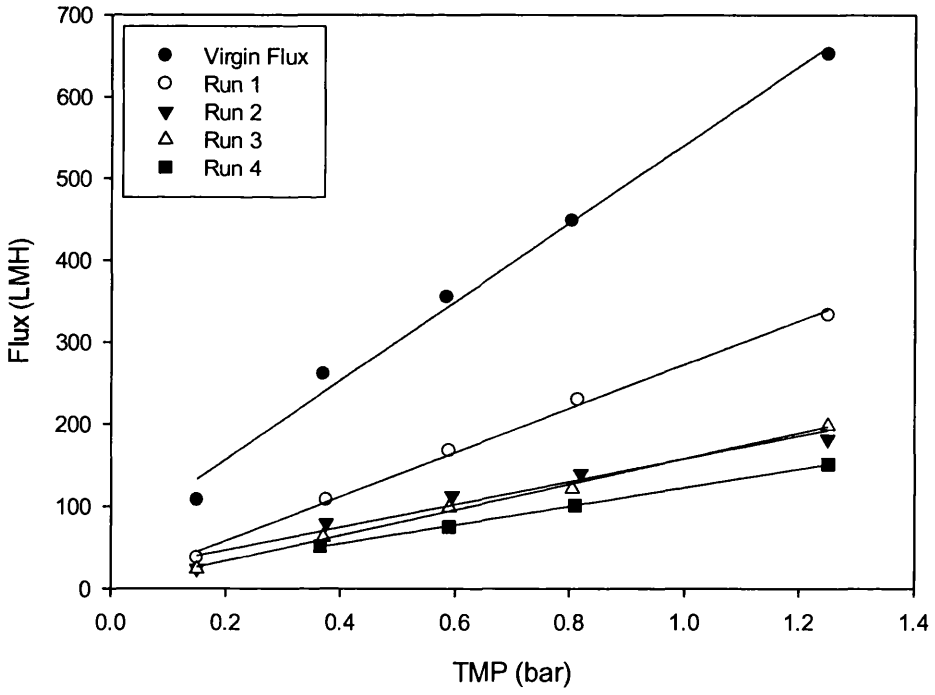

Figure 6.2.4: Flux versus TMP for various runs during pilot scale microfiltration

Table 6.2.5: Results of pilot scale microfiltration process

Run	Stage	Sample	Pressure (bar)	Processing Flux (LMH/bar)	Processing Percentage Clean Water Flux (%)	Percentage Original Flux at 0.8 bar TMP (%)
1	MF	Industrial Potato Water	1.00	91.5	27.4	50.5
2	MF	Extraction Water	1.00	44.6	16.3	30.6
3	MF	Extraction Water	0.50	72.0	41.0	27.1
4	MF	Extraction Water	1.50	46.2	28.8	22.5

The processing flux rates shown in Table 6.2.8 are highly variable. The highest flux of 91.5 LMH/bar was obtained through the processing of the potato water, however this was the first run undertaken therefore the membrane had minimal fouling at the start of the process. The lowest flux rates were observed during run 2 and 4 where pressures of 1.0 and 1.5 bar were used respectively, possibly suggesting that a higher pressure leads to a higher degree of foulant deposition on the membrane surface. This hypothesis is backed up by the observation that the process flux as a percentage of the clean water flux was 16.3 and 28.8 % for runs 2 and 4 respectively. What is surprising however is that the flux percentage of the clean water flux is higher for the material processed at 1.5 bar suggesting lower fouling observed at the higher pressure. However, it must be appreciated that although the same feed is processed throughout runs 2, 3 and 4, any slight variations may result in different membrane performance, and furthermore it is impossible to fully appreciate the significance of pressure on fouling due to each run being run consecutively on the membrane.

In order to recover flux lost during processing various membrane cleaning protocols were investigated. The majority of the fouling observed during the microfiltration process was due to starch deposition on the membrane surface. The properties of

starch result in a glue like layer on the membrane surface which results in other compounds becoming trapped upstream as well as a significant change in the flux properties of the membrane. Often sodium hydroxide is used in order to recover lost membrane flux, however when a cleaning protocol using NaOH was used the flux recovery was fairly poor. NaOH is often used for the removal of fatty deposits on the membrane surface and therefore had very little effect on the degradation of starch. Therefore, an alternative cleaning regime was required. Starch is only slightly soluble in cold water, and the solubility only slightly increases with hot water. Furthermore starch is only partially soluble in most common solvents. The cleaning regimes studied investigated the potential of starch gelatinization where starch is heated to above the gelatinization temperature (approx 55 - 60°C (Singh et al., 2008)) in the presence of water, which leads to the irreversible solubilisation of the starch molecule. Therefore a cleaning regime was designed consisting of a 70°C water wash at 1.5 bar for 30 minutes in recycle mode, followed by a 30 minute 0.25M NaOH at 25°C wash followed by a 30 minute 70°C water wash where the system water input matched the system output i.e. fresh hot water continually passing the membrane surface. The direct comparison of the flux recovery values obtained using the various cleaning methods is not possible due to varying quantity of fouling observed in between each clean, however the cleaning method described obtained a flux recovery of 112% compared to the water flux at a TMP of 0.8 bar prior to cleaning. The cleaning stage may be further optimised, however it is believed that any future micro filtrations of potato extracts should be conducted using ceramic membranes as opposed to hollow fibre membranes where a much more aggressive cleaning regime may be undertaken. A further avenue of research is the use of amylase in order to biologically degrade the starch into simpler sugars in order to obtain a better flux recovery. Prior to full commercialisation of any membrane process the cleaning strategy to be employed must be fully evaluated. The work undertaken here briefly considered membrane cleaning, however, the main aim of this work was to produce a feasibility study for the use of membrane technology for the recovery of a biological molecule from waste potato peel. The initial cleaning strategies investigated displayed an overall flux decline despite the use of different cleaning techniques. Overall no clear conclusions may be drawn

from the MF cleaning methodologies investigated as there was no standardisation of the fouled membrane starting point. The optimisation of membrane cleaning should be thoroughly considered as a dramatic flux decline that is unrecoverable would result in an unfeasible process which would discard any potential benefits of using membrane technology.

Total Organic Carbon (TOC) reduction during microfiltration is shown in Table 6.2.6. The MF process achieved an average TOC reduction of 25.1 %. However, the reduction observed during processing at 1.0 bar was significantly lower than the other runs with a TOC reduction of 8 %. The results obtained show an average reduction of 14 g in carbonaceous material across the MF process.

Table 6.2.6: TOC reduction observed during pilot scale microfiltration

Microfiltration	**Feed TOC**	**Permeate TOC**	**TOC Reduction**
Sample	**(mg)**	**(mg)**	**(%)**
0.5 bar	62460	45080	27.8
1.0 bar	68220	62748	8.0
1.5 bar	57420	39330	31.5
Potato Water	50250	33632	33.1

The reduction in sugar and protein by the microfiltration process was studied and the results are displayed in Table 6.2.7:

Table 6.2.7: Sugar and protein reduction observed during pilot scale microfiltration and nanofiltration

	Sugar (g)			Protein (g)		
Sample	**Feed**	**Permeate**	**% Reduction**	**Feed**	**Permeate**	**% Reduction**
MF - Potato Water	4.3	0.1	98.5	25.1	0.7	97.2
MF 0.5 bar	78.8	60.3	23.5	23.8	11.0	53.8
MF 1.0 bar	100.6	61.3	39.0	24.8	10.6	57.4
MF 1.5 bar	88.6	62.5	29.4	27.4	9.8	64.1
NF - 10 bar	22.3	1.0	95.7	14.4	3.9	72.8
NF - 20 bar	29.8	2.1	93.0	20.5	7.3	64.5
NF - Potato Water	3.2	0.4	86.7	14.3	6.1	57.2

The potato water showed a 98.5% reduction in sugar across the microfiltration process. This value is exceptionally high for a microfiltration process; however the feed contained the lowest quantity of sugar in the feed. The high sugar reduction value obtained may be explained if the largest percentage of sugar present was in the form of starch which is removed by the microfiltration process. The processing of the peel extracts resulted in sugar reduction values of 23.5, 39.0 and 29.4 % at 0.5, 1.0 and 1.5 bar respectively. Interestingly, despite the varying levels of sugar in the feed the three permeates all contained approximately 61 g of sugar in the same volume suggesting that the sugar reduction is attributed to the starch being removed. The protein reduction is again largest with the potato water with a reduction of 97.2 %, however in this case the feed contained a similar quantity of protein as the peel extracts. The three peel extracts again had a similar level of protein in the permeates however, in contrast to the sugar results showed an increase in protein rejection with increasing microfiltration operating pressure, increasing from 53.8% at 0.5 bar to 64.1% at 1.5 bar.

Nanofiltration and Reverse Osmosis

The pure water and processing fluxes for the pilot NF process are shown in Figure 6.2.5 and Table 6.2.8.

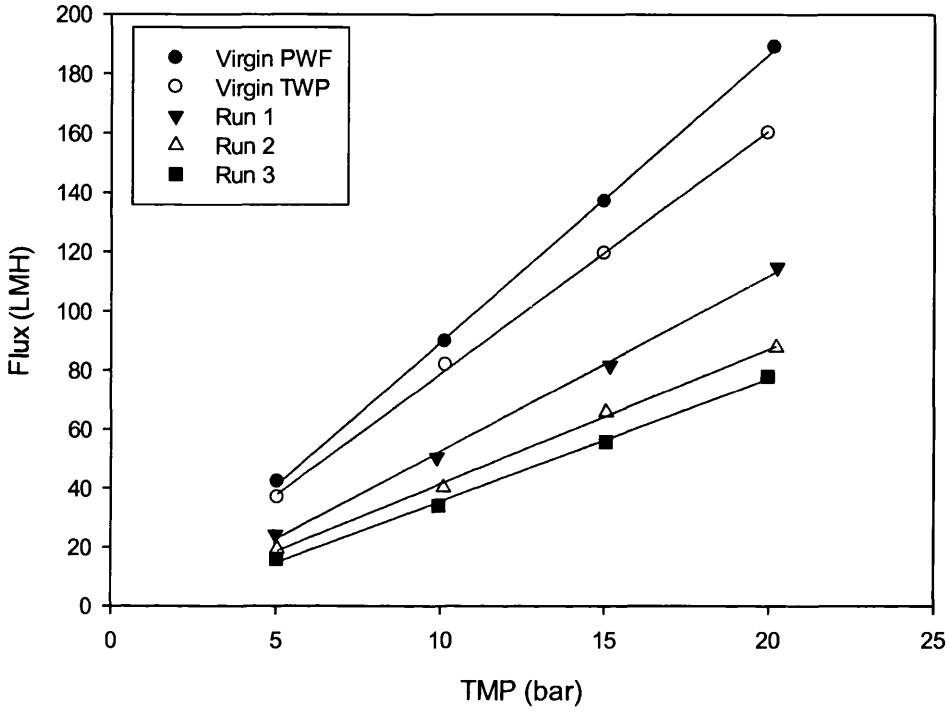

Figure 6.2.5: Flux versus TMP for various runs during pilot scale nanofiltration

Table 6.2.8: Results of pilot scale nanofiltration process

Run	Stage	Sample	Pressure (bar)	Flux (LMH/bar)	Sample Percentage Clean Water Flux (%)	Percentage Original Flux at 20 bar TMP (%)
1	NF	Potato Water	10	4.3	59.5	71.6
2	NF	Extraction Feed	10	2.8	53.1	54.9
3	NF	Extraction Feed	20	2.2	53.2	49.3
-	RO	Reverse Osmosis	20	0.91	-	-

The initial pure water flux of the virgin membrane was 8.97 LMH/bar obtained using ultra pure water. Due to the volumes required the use of ultra pure water on an industrial scale is costly and therefore unfeasible, especially when the required purity is unnecessary. This resulted in clean tap water being used for all water flux experiments throughout the pilot scale experimentation. The effect of using tap water as opposed to ultra-pure water must be considered. Tap water contains much higher levels of ions than ultra-pure water and therefore alters the extraction potential of the water i.e. less of extraction potential. The mineral content of tap water may result in an alteration of the membrane properties when compared to ultra-pure water. Furthermore the mineral content of the tap water may vary slightly from day to day, however this is likely to replicate an industrial extraction. The effect of tap water on the extraction and separation performance may be evaluated by comparison to identical extraction and separations using ultra-pure water. Such experiments would allow the true effect of using tap water to be identified. The pure tap water flux obtained for the virgin membrane was 7.84 LMH/bar which equals a 13 % reduction when compared to the ultra pure water originally used, a reduction which is expected due to the presence of salts in mains water. Figure 5.2.5 shows that the pure water flux after each subsequent processing stage decreases displaying an overall flux decline 50 % after all the processing stages. The first run where the potato water was processed experienced a 28.4 % flux decline followed by a 45.1 and 50.7 % flux decline for runs two and three (extract processing at 10 and 20 bar respectively). Similarly a decline in flux is observed with each subsequent processing run where a flux of 4.3 LMH/bar is seen for the potato water, followed by a flux of 2.8 and 2.2 LMH/bar for the 10 and 20 bar (Run 2 and 3) operating conditions respectively. The significant flux decline is in agreement with the data obtained from the laboratory scale experiments, where an average flux of 3.3 LMH/bar was seen, however this value was obtained through experimentation once on each membrane as opposed to subsequent experiments as is the case with the pilot scale study. The fluxes obtained for runs 1, 2 and 3 were 59.5, 53.1 and 53.2 % of the pure water flux values, values which are higher than the values obtained in the laboratory scale equipment where the largest processing pure flux value was 43.3 %. The direct comparison between the two process scales

acts as a guide rather than a definitive answer due to the significant variation in the feed introduced to the processes. Further research would be required in order to investigate the flux decline with processing time. The continuation of flux decline with each processing step would suggest that either the selected nanofiltration membrane is unsuitable or that further work is required in order to remove the foulant prior to the nanofiltration stage.

The large flux decline observed on the pilot scale would render the process unfeasible over an extended period due to the continual cost of replacing the membranes. The cleaning regimes investigated attempted to avoid the extensive use of chemical cleaning agents due to possible contamination of the final product in a subsequent run. For this reason a 30 minute water flush was conducted at a high cross flow rate of 42.5 litres/min followed by a one hour 0.005 w% NaOH wash at 25°C. The system was then flushed with pure water until the system pH returned to neutral. The overall results of the NF process showed a flux decline, and although the cleaning protocol displayed a flux recovery the net loss of flux with subsequent results is of concern. Therefore similarly to the MF further work is required in order to optimise the nanofiltration cleaning method either through the prevention/reduction of fouling during operation or through the use of a more significant cleaning stage such as the use of surfactants. Due to the nature of the NF process it may be necessary to implement an additional pre-filtration stage to the process (such as UF) in order to reduce the amount of fouling the membranes observe. The nature of the fouling materials would need to be considered in greater depth as this greatly affects the chemicals used in order to restore the membrane flux. Similarly to the MF study performed the effect of membrane cleaning would need to be further considered in order to produce a feasible commercial process.

The TOC measurements for the nanofiltration stage are shown in Table 6.2.9.

Table 6.2.9: TOC reduction observed during pilot scale nanofiltration

Sample	Pressure (bar)	Feed TOC (mg)	Permeate TOC (mg)	TOC Reduction (%)
Potato Water	10	33620	2380	92.9
Extraction Feed	10	42990	23384	45.6
Extraction Feed	20	66750	47772	28.4
Reverse Osmosis	20	105632	20230	80.8

The values obtained are varied with the highest TOC reduction seen in the processing of the potato water. A TOC reduction of 45.36 % was obtained for the extract processed at 10 bar and a reduction of 28.4% for the extract processed at 20 bar. These values suggest that show that as the feed TOC increases the overall TOC reduction decreases, as is often seen with nanofiltration when the feed concentration is high. Direct comparison between TOC reduction figures is not possible due to the variation in the feed TOC, which therefore prevents a relationship between operating conditions and TOC reduction to be observed. The results obtained would suggest that the use of a synthetic feed would be beneficial in order to have more process control.

The pilot scale sugar and protein rejection results are shown in Table 6.2.7. The nanofiltration process produced a sugar reduction of 95.7 and 93 % at 10 and 20 bar respectively when processing the peel extract. The values obtained are in the range expected for a nanofiltration process. It is not possible to directly compare the sugar reduction values obtained at the pilot scale to the value obtained during the laboratory study due to the vast difference between the process feeds, however the values are within the same range. The potato water exhibited a sugar reduction of 86.7% which is slightly lower than the peel extract samples. This value is slightly lower than expected due to the feed concentration being the lowest of all the nanofiltration feed samples. The nanofiltration protein reduction values are

slightly lower than would be expected with values ranging from 57.2 to 72.8%, however analysis by GC (GC chromatograms in appendix A5) displayed the presence of amino acids which would explain the reduction values observed. Full observation of the chromatograms display the presence of amino acids which are constituent of longer chain proteins. A protein reduction of 72.8 and 64.5% was observed at 10 and 20 bar respectively for the peel extract samples, while the potato water displayed a reduction of 57.2%.

Initially the intention was to reduce the volume of the product prior to drying using a large rotary evaporator similar to the laboratory scale; however this is not feasible at a large scale. Reverse osmosis (RO) was therefore used in order to reduce the permeate volume prior to freeze drying. The nanofiltration process produced a combined product volume of 140 litres which results in a final product volume after RO of 15.35 litres which corresponds to a 89% volume reduction. The RO process observed a flux of 0.91 LMH/bar and displayed no significant flux decline over the volume processed suggesting minimal fouling. The insignificant level of fouling is expected as the feed into the RO process is the permeate of the NF process and therefore is very low in fouling materials such as proteins and starch. A TOC reduction of 80.8% was observed in the RO process suggesting the removal of a large quantity of carbonaceous material, although ideally this figure would be higher (>95%) in order to retain all the compounds in the RO retentate, i.e. dewater only. The RO rejection being so low suggests that a significant quantity of carbonaceous material is being permeated through the membrane. The rejection of the RO process is dependent on the quantity of carbonaceous material in the feed i.e. the higher the feed TOC the lower the rejection. The results obtained would suggest that if RO is to be used as the process for concentration a double pass RO would be required in order to obtain maximum compound recovery. A similar system was employed by Vourch et al. (2005) where a double pass RO system was used in order to further purify dairy industry wastewaters to provide a permeate of drinking water standard.

Mass Balance

The efficient extraction and purification of calystegine compounds are the main purpose of this feasibility study and therefore a mass balance was performed over the pilot scale using the semi- quantitative GC results (Chromatograms in appendix A5). The many iterations performed throughout this design process notice highly variable results in the quantification at the laboratory scale due to the low concentration of calystegine present and the detection limits of the equipment. The calystegine concentration was higher in the pilot scale experiments and therefore made the detection and quantification more reliable, however some of the samples analysed were again at the detection limit of the equipment. Therefore, the mass balance developed has a higher than desired error, however the values obtained are acceptable for the appreciation of where the majority of calystegine enters and exits the process.

Table 6.2.10 shows the mass balance of the calystegine compounds over the entire pilot scale process. The mass balance shown in Table 6.2.10 has measured the concentration of calystegines in both the feed and permeate. The levels of calystegines present in the retentate were measured and found to be close to or above the equipment detection limit. The work, should it be repeated, would require the mass balance to be closed through the measurement of the sample retentates in order to more accurately determine the process mass efficiency. The analytical work performed was undertaken once due to the quantity of materials consumed and the availability of analytical equipment (method proprietary to PhytoQuest Ltd). The running of samples once only is not ideal, however, due to the constraints and nature of the project this was mandatory. Nevertheless, as the analysis was performed by GC-MS with high accuracy the results were deemed acceptable for the intended purpose. The results of the mass balance show that 132.1 mg of calystegines entered the process as feed with 49.3 mg of the initial mass being calystegine B_2. The results of the microfiltration process are rather surprising as 66.5 % of the initial mass of calystegine that entered the process is lost at this stage. In a real industrial process this value would be unacceptably high as nearly two-thirds of the final product is lost in the pre-filtration stage. Overall a

significant quantity of each calystegine is lost during the microfiltration stage. Although interestingly, the extract processed at 0.5 bar displayed the greatest loss of mass during microfiltration with an average yield of 10.3%. The overall highest yield was obtained through processing at 1.5 bar with an average yield of 63.0%. This would suggest that a higher operating pressure helps reduce the losses observed during the prefiltration stage. The processing of the potato water obtained an average yield of 45.5% which is again lower than desired. The low yields may be attributed to the high degree of fouling observed on the membrane when processing. Furthermore there is a possibility that the calystegine may bind to the starch compounds present in the feed and become trapped in the microfiltration retentate. There is a possibility that the calystegine compounds are becoming bound to the starch compounds or being prevented from permeating by a starch layer forming on the membrane surface. In order for the process to be feasible the possibility that the calystegines are being bound to the starch needs to be fully investigated. In order to investigate the binding of calystegines the MF retentate should be subjected to diafiltration in order to retain only the starch compounds. The starch fraction retained by the membrane can then be tested for the presence of calystegine compounds. The process of diafiltering the MF retentate should allow all of the calystegines to be permeated and hence should there be any calystegines detected then this would confirm the suspected binding of calystegines to the starch compounds present.

Table 6.2.10: Calystegine mass balance over pilot scale process

	A3			A5			B2			B4		
Sample Name	**Feed**	**Permeate**	**Yield**	**Feed**	**Permeate**	**Yield**	**Feed**	**Permeate**	**Yield**	**Feed**	**Permeate**	**Yield**
	(mg)	**(mg)**	**(%)**	**(mg)**	**(mg)**	**(%)**	**(mg)**	**(mg)**	**(%)**	**(mg)**	**(mg)**	**(%)**
MF - Potato Water	9.4	4.2	44.8	4.1	2.3	55.0	13.1	5.8	44.6	2.5	0.9	37.6
MF - 0.5 bar	19.9	1.9	9.5	10.3	1.2	11.6	20.8	1.9	9.1	2.3	0.3	11.1
MF - 1.0 bar	13.0	6.3	48.4	10.6	4.2	39.5	11.4	6.7	59.2	1.3	0.6	51.3
MF - 1.5 bar	5.0	2.5	50.8	3.9	1.3	33.5	4.1	3.6	87.3	0.5	0.4	80.5
NF - 10 bar	5.9	3.6	61.7	4.9	1.8	37.4	6.9	4.0	58.5	0.6	0.5	87.1
NF - 20 bar	8.9	7.1	79.6	7.3	4.7	63.7	10.3	8.8	85.4	0.9	0.9	103.4
NF - Potato Water	4.2	1.1	27.1	2.3	0.5	21.8	5.8	1.7	28.5	0.9	0.2	26.1
RO	8.3			3.8			11.5			1.0		

	A3		A5		B2		B4	
(mg)	**IN**	**OUT**	**IN**	**OUT**	**IN**	**OUT**	**IN**	**OUT**
MF Mass	47.3	14.9	28.9	8.9	49.3	18.0	6.6	2.3
NF Mass	19.0	11.9	14.5	7.0	23.0	14.5	2.5	1.7

(mg)	**IN**	**OUT**
TOTALS MF	132.1	44.2
TOTALS NF	59.0	35.1

(mg)	**IN**	**OUT**	**Yield (%)**
Calystegine B2 Mass	49.3	14.4	29.2

Overall these findings suggest the need to greatly optimise the prefiltration stage of the process, through both reducing the fouling of the membrane and improving the permeation of the calystegine compounds. The polymeric microfiltration membrane used in this study should be replaced by a ceramic membrane allowing the use of a much more intensive cleaning regime, along with the use of diafiltration or constant volume filtration which should allow higher calystegine yields to be obtained.

59.0 mg of calystegines entered the nanofiltration process as feed of which 23.0 mg was calystegine B_2. The combined permeates of the NF stage contained 35.1 mg of which 14.5 mg was calystegine B_2. The yield of the nanofiltration stage was 59.5 % a value which is acceptable for the first pilot scale interaction, however it would be hoped that this value may be increased to above 90% with correct optimisation of the nanofiltration stage. In order to increase the yield of the nanofiltration stage it would be necessary to investigate the implementation of a tight UF stage in order to attempt to permeate all calystegines prior to concentration using a NF membrane. Interestingly the theoretical rejection of calystegine B_2 at 20 bar was 71% suggesting that only 29% of the calystegine in the feed would be permeated. However this is not the case and can be attributed to the effects of pore size distribution where the membrane MWCO is in the range of 400 - 1000 Da. Furthermore the theoretical rejection values quoted are based on real rejections where the experimental values obtained do not account for concentration polarisation and fouling effects. The highest yield of calystegines was obtained through processing the extract at 20 bar with calystegine B_2 displaying a yield of 85.4%. Interestingly, the lowest yield was obtained was obtained when processing the potato water, possibly due to the lower calystegine concentrations in the feed suggesting that the rejection is higher. The large variation dependent on the feed concentration is of concern as the results suggest that the overall yield and efficiency of the nanofiltration process is highly dependent on the feed calystegine content. Therefore, the NF process would undoubtedly benefit from significant optimisation in order to either fully retain or fully permeate the calystegine compounds.

The overall process yield of calystegine B_2 was 29.2%. The mass of calystegine B_2 obtained from the process was 14 mg, derived from 20 Kg of potato peel. This value results in a mass of 0.72 mg/Kg of potato peel. In order to contextualise the yield obtained from this experimental work the results should be compared to other authors who have extracted calystegines from potato peels. This study does vary from the other works present in the literature in that this work was undertaken on a much larger scale and separated and purified using membrane technology avoiding the use of organic solvents. Friedman et al. (2003) obtained a value of 141 mg/kg of calystegine B_2 which is significantly higher than the value obtained in this study. However, the work undertaken by Friedman et al. (2003) was for the measurement of calystegine levels within potato peel unlike this study where calystegines are extracted for other uses. Similarly the work undertaken by Keiner and Dräger (2000) also measured the calystegine content within potato plants reporting similar values to Friedman et al. (2003). The work undertaken in this study did not simply measure maximum concentration but attempted to extract the available calystegines in order for the compounds to be studied and utilised further, and as such direct comparison of values obtained is not applicable. Overall, the results of this work suggest that the extraction and purification of calystegines from potato peel waste using membrane technology is technically feasible. The step-wise optimisation of each stage of the processes should see the overall process yield dramatically increase.

6.2.4 Further Development

As with any feasibility study more questions than answers are generated throughout the process, with this study no exception. The study undertaken has provided a once through attempt at producing a calystegine extract on a pilot scale, based on results found at a laboratory scale. The initial findings suggest that the production of a calystegine extract from waste potato peel using a solvent free extraction followed by purification using membrane technology is feasible. However, the results obtained over the study period have resulted in a number of areas where further research is required in order to optimise the process. Due to

the linear nature process of the process the optimisation should be undertaken sequentially and as such should be split into distinct sections.

A recommendation for these sections are as follows:

Analysis

The analysis undertaken has provided an excellent starting point for the appreciation of the designed process, however due to the size based separation methods used there is potential of extracting and recovering other valuable compounds such as starch simultaneously. Therefore the careful analysis of each stream is highly important to add further value to the designed process. Furthermore, the semi-quantitative calystegine method used thus far requires modification in order to improve the mass balance produced across the system. In addition, for safety the potato extract should be analysed for the presence of glycoalkaloids.

Extraction

- Research extraction time, paying particular attention to the pre-rinse in order to remove fast extracting sugars and proteins.
- Investigate the possibility of using ethanol (depending on proposed product use) to aid extraction of calystegines.
- Investigate the removal of starch prior to entry into membrane systems; possibly investigate coagulation, or other removal techniques.

Solids-removal

- The removal of all solids prior to entry into the membrane process is essential therefore a solid removal technique is essential. Further investigations should consider the use of an in-line filter or due to the large solids loading a possible sedimentation or sieving process may be necessary.

Pre-filtration

The work thus far has concentrated on the use of a microfiltration prefiltration step; however the results obtained from the study suggest that not all the calystegine compounds are permeated through the microfiltration membrane. Furthermore the nanofiltration retains a large percentage of the calystegines along with many other compounds (sugars, proteins, etc.) resulting in an extremely large range of MW in the NF retentate fraction. Therefore, the implementation of an effective ultrafiltration stage would allow greater fractionation and higher purity products. Further pre-filtration research required includes:

- Optimisation of membrane (pore size, membrane material, operating conditions).
- Investigation of membrane cleaning regimes.
- The use of dia-filtration in order to increase yield.
- Implementation of an ultrafiltration stage to further fractionate and purify process streams.

Nanofiltration

The nanofiltration membrane separation is the most vital part of the process and plays a key role in determining the overall purity and yield of the process. The original process attempted to permeate the calystegine compounds through the nanofiltration membrane while retaining the majority of the sugars and proteins. The results obtained from the pilot scale experiments suggest that the use of nanofiltration is feasible however much optimisation is required. The areas that require optimisation include:

- Optimisation of membrane pore size/ MWCO.
- Optimisation of membrane operating conditions (Pressure, cross-flow rates).
- Investigation of using dia-filtration in order to increase process yield.
- The application of cleaning regimes to extend membrane life.
- Optimisation of nanofiltration along with ultrafiltration in order to produce a purer fraction.

Reverse Osmosis

- Optimisation of reverse osmosis process, investigation of multi-stage process in order to maximise recovery.
- Investigate maximum obtainable volume reduction in order to reduce loading on drying stage (Final concentration is dependent of required application).

Product Purification and Drying

Further purification and drying would not normally be necessary should the product be sued as a food additive, however, should a purer fraction be required ion exchange should be used. The concentration with reverse osmosis would allow a much lower solvent volume to be used for the ion exchange process, reducing the environmental, safety and cost impact of the final process. In order to assess the true process parameters the optimal resin loading, application time and overall column efficiency should be studied. Additionally investigation of enzymatic methods to reduce simple sugars in the final product may be investigated.

The drying of the product would be unnecessary should a liquid extract be required, although should a dry fraction be required drying methods should be investigated in order to preserve product integrity. Freeze drying is an obvious candidate due to the gently nature of the drying process, though spray drying and vacuum drying should be investigated in order to determine the cheapest and most efficient drying method.

Product Assays

The biological properties of the calystegines should be thoroughly investigated both as a concentrated extract and as a pure product. Calystegines have been previously studied for their glucosidase inhibition, as well as their antiviral and antimicrobial properties. The extraction and purification of large quantities of calystegines from waste potato peel will allow the biological properties to be fully investigated.

6.2.5 Conclusion

Overall, the feasibility study undertaken for the extraction and purification of calystegines from waste potato peel has been successful. The results obtained suggest that the extraction and purification of calystegines from waste potato peel using a solvent free membrane process is possible. The process requires optimisation, with each stage requiring further work both individually and as part of the whole process. The pilot scale trial produced 14 mg of calystegine B_2 from 20 Kg of waste potato peel at an overall process yield of 29%. Further process improvements should see this figure rise significantly. The possible improvements are listed above, however this list is not exhaustive, and as with any improvement process further avenues of research interest will arise. To summarise the process designed is feasible for the large scale extraction and purification of calystegines from waste potato peel. The work undertaken in this study is the first to successfully isolate calystegines in any significant quantities. Despite the obvious improvements that may be implemented to the extraction and purification process the initial steps undertaken allow calystegines to be further investigated as potential new bioactive compounds for a variety of new applications. Furthermore the process designed adds a significant value to that of waste potatoes.

7.0 Overall Discussion and Conclusions

The objective of this thesis was to investigate the use of membrane technology, and in particular nanofiltration, for the improved separation, purification and concentration of bioactive compounds. Nanofiltration, although a relatively young technology, is now firmly established in the pre-treatment of seawater desalination; used both for the prevention of scaling and load reduction of the reverse osmosis membranes. The growth of nanofiltration has seen the development of a number of theoretical predictive models to help promote the understanding of the separation mechanisms involved in this highly complex process.

NF membranes are increasingly employed as a viable alternative to more established separation processes in a diverse range of industries, in particular for the concentration and separation of small solutes. The growth of 'Nanotechnology' will undoubtedly see further growth of nanofiltration although, as the current literature suggests, the true potential is not yet being exploited. The literature review presented in chapter 2 suggests that most nanofiltration applications are either for the treatment of a variety of waste waters or for general concentration applications, due primarily to the lower cost of NF when compared to RO. These applications are of vital importance and, along with the pre-treatment of seawater for desalination, will ensure the long term application of nanofiltration technology. NF also has the potential for more niche applications particularly in the search for novel bioactives, a notion this thesis has attempted to explore.

The use of laboratory scale frontal filtration equipment is an essential pre-requisite for both theoretical understanding and process development. The scale of the apparatus allows for the experimentation with real industrial materials, and sufficient experimental data has been reported to suggest that the results obtained from laboratory scale apparatus is representative of full scale industrial equipment. For the accurate analysis of the laboratory scale data, the mass transfer effects of the membrane equipment must be known. An experimental analysis of the mass transfer characteristics of the laboratory scale dead-end filtration cell used in all

experimental work showed that concentration polarisation effects may be largely neglected in the Amicon cell, whilst the Sterlitech and Membranology cells both exhibit significant concentration polarisation as a result of the significantly higher pressures employed (which leads to higher flux rates). Optimisation of stirring is essential for the reduction of mass transfer as well as for the optimisation of the mass transfer coefficients. The work undertaken demonstrated that a maximum stirrer speed of 300 rpm is most suitable for the membrane cells studied, with higher stirrer speeds unobtainable due to equipment limitations and vortexing. The lower operating pressures and hence flux rates associated with the Amicon cell resulting in minimal concentration polarisation. The higher pressures and flux rates obtained with the Sterlitech and Membranology cells result in significant concentration polarisation with both cells exhibiting similar mass transfer, however the larger surface area of the Membranology cell results in better flux performance. Comparison of the mass transfer data obtained from the three membrane cells, to that determined from theoretical correlations was found to inaccurately predict the mass transfer for nanofiltration systems, and as such, a modification of the mass transfer correlation was proposed.

The transport of a solute through a pore results in both convective and diffusive hydrodynamic drag forces, the values of which are widely used in the theoretical prediction of the nanofiltration separation process. The theoretical descriptions found in the literature are often algebraically derived through finite analysis techniques and as such these factors have not been experimentally derived. The physical determination of membrane pore size and PEG solute size combined with experimental PEG rejection experiments allowed the hindrance factors to be experimentally derived. This enabled the validity of the theoretical correlations to be determined. The study performed established a pore size of 1.075 nm for the Nadir 4000 MWCO membrane.. The derived values obtained for the hindrance factors were found to be in good agreement with the theoretical predictions which suggests that the correlations found throughout the literature are accurate enough for the calculation of the hindrance factors describing the hydrodynamic drag forces experienced by a solute in a nanopore.

The growing use of membranes for the desalination of seawater for the production of potable water has seen an increased demand for better understanding of the membrane processes. Accurate methods for the design, development and scale-up of salt water desalination plants are vital for developing cost effective and sustainable plants. The study undertaken examined the use of the extended Nernst-Planck equation for predicting the separation properties of single salt solutions ranging in concentration from very dilute to a concentration similar to that of seawater. The experimental results highlighted shortcomings when applied to highly concentrated solutions, suggesting a lack of understanding of the physical properties and physico-chemical phenomena inside nanopores. The isoelectric point of the Desal DK was found to be at pH4.. The effect of dielectric exclusion was further investigated through conducting the experiments at the membrane's isoelectric point with the results obtained suggesting that screening of the dielectric behaviour may be occurring at higher salt concentrations. Salt rejection over a variety of concentrations varied from almost 100% at a concentration of 0.001M to 60% at 0.6M at the membranes isoelectric point. This screening phenomenon is not properly accounted for using the Born model.

The applications of membrane technology are ever expanding with new areas being continually explored. An area of interest is the application of membrane technology for biological separations and in particular bioactive separations. Bioactive molecules often in the nano range (MW<1000) and are therefore suitable for separation by nanofiltration.

The use of nanofiltration for the separation and purification of fungal bioactives has been successfully explored. Entomopathogenic fungi are known to be an excellent source of bioactive compounds with potential uses from bio-control agents to medicines. The extraction of the bioactive compounds from fermentation broths often uses solvent extraction techniques. Through the use of membrane technology the process devised avoids the separation bias, safety and cost aspects of organic solvents. The process developed was tested with a variety of fungal (*Metarhizium*) strains and media, displaying excellent recovery of the bioactive compounds, as

well as indicating the robustness of the process. The two membranes studied despite the differing MWCO displayed similar rejections of the bioactives (47.2 and 52.9% rejection of destruxin E for the NTR-7450 and NF200 membranes respectively) suggesting the separation is partially governed by the fouling layer present. Nanofiltration as a separation method based on both size and charge may allow nanoscale metabolites to be detected that may otherwise be lost through a biased separation technique. Furthermore, the results contained within this thesis have demonstrated that products obtained from the membrane based process have a higher bioactivity that those obtained by the traditional solvent extraction process. The mortality of the mosquitoes studied was higher at almost 80% when subjected to the retentate of the nanofiltration when compared to the approximately 35% mortality observed with the solvent extraction product.

The extraction and purification of calystegines has been an area of interest for the last 30 years. One factor which has impeded the large scale evaluation of their properties have been the quantity at which the compounds can be extracted. Investigations into the potential bioactive properties of calystegines is only limited by the design of an effective extraction and purification technique. Potato plants are known to contain calystegines with the highest levels being found in the skin and peel. Potato peel is a low value waste compound and as such offers a virtually unlimited source of the compounds. The feasibility of membrane technology for the concentration and purification of calystegines was examined in this experimental work. An initial composition study was conducted in order to determine the optimal storage and extraction conditions required to obtain the maximum calystegine concentration. The storage study undertaken showed that storing peel does not significantly increase the calystegine levels in the peel and therefore processing should proceed immediately. The initial concentration of calystegines in the potatoes was approximately 40 mg/kg however after the eight week storage the calystegine level dropped in all storage conditions except the whole potatoes stored under cold light conditions. The extraction study resulted in the optimal extraction period being determined as 48 hours and showed that the sugar and proteins present in the peel are quickly extracted suggesting an initial peel wash

would remove some of the sugars and proteins which would produce a purer process feed. The second part of the study was concerned with the development of a membrane based process for the separation and purification of the calystegines. The study undertaken optimised the process on a laboratory scale by applying microfiltration followed by nanofiltration. The peel obtained from an industrial processing facility varied significantly and as such the process experienced greater fouling than that observed at the laboratory scale. The process undertaken produced 14 mg of calystegine B_2 from 20 kg of waste potato peel at an overall process yield of 29%. The process designed was shown to be feasible for the extraction, separation and purification of calystegines from waste potato peel, although the results suggest process optimisation needs further study. The process implemented was shown to be feasible for the purification of calystegines on an industrial scale.

In summary, the studies undertaken within this thesis have highlighted that membrane technology, and in particular nanofiltration, has great potential in the separation and purification of bioactive compounds from natural sources. Membrane technology however does not provide all the answers. Membrane technology has been previously shown to be an effective method for the separation of multi-component mixtures (2-4). When applied to mixtures which contain a far greater number of compounds the separation becomes much more difficult, particularly when the compound of interest is in low concentration (Calystegines, Destruxins and Cytochalasin in this work). Membrane technology therefore must be thought of as another tool in the arsenal of a separation scientist/engineer and should be combined with other technologies. The work undertaken has shown nanofiltration technology to be applicable for laboratory scale separations (fungal bioactives) as well as on the large scale (recovery of calystegines), particularly for the production of high concentration extracts. Interestingly, the experimental modelling work undertaken in this thesis (Chapter 4) has highlighted the need for greater knowledge for the modelling of 'real' industrial chemical solutions which would suggest that the theoretical understanding of the much more complex biological solutions is not yet complete. The work contained within this thesis has

shown membrane technology to be an excellent method for primary isolation. The work has shown the feasibility of the technology in producing a concentrated extract, however for higher purity the technology may require an additional high resolution step such as ion exchange or chromatography. The purpose of this study was to investigate the potential of membrane technology for primary separation and concentration of bioactives from natural sources, and this aim has been achieved. Further work on combining the technology with high resolution separation techniques may be worthwhile should high purity extracts be required. The work presented within this thesis will enable membrane technology and in particular nanofiltration to be implemented, or at least considered, for the separation, purification and concentration of small bioactives from natural sources.

8.0 Recommendations

The following recommendations will further develop the work presented in this thesis:

- Further investigate the proposed mass transfer correlation using a variety of nanofiltration membranes and different solutes to further improve the validity of the model proposed.
- Investigate standardisation of membrane characterisation techniques. Further investigate and characterise a number of nanofiltration membranes using a variety of techniques in order to determine optimum characterisation methods for application across the membrane industry.
- Further investigate the applicability of the theoretical descriptions of hindrance factors by using a variety of solute molecular sizes, concentrations and membrane pore sizes. Experiment across the whole range of nanofiltration membrane pore sizes to validate the theoretical descriptions of hindrance factors.
- Investigate the effect of dielectric exclusion further by repeating salt rejection experiments with divalent ions in an attempt to investigate whether the theoretical models require the inclusion of a concentration effect.
- Further improve the fungal filtration process through membrane optimisation to achieve higher purity, recovery and concentration of the fungal bioactives studied. Investigate the potential of using membrane technology as a rapid screening technique for a range of biological compounds for the rapid detection of potentially bioactive compounds.
- Further optimise the extraction of calystegines from potato peel waste by investigating the correct membrane selection on a pilot scale, and subsequently test the bioactivity of the fractions obtained. Investigate the product concentration and purity required in order to produce the required level of bioactivity. Investigate the potential for producing a standardised pure product using membrane technology.

9.0 Appendices

Appendix A1: Mass transfer study of three commercial frontal filtration membrane systems and comparisons of experimentally derived mass transfer values to theoretical correlations

3.5.1.1 Introduction

The performance of membrane applications is normally evaluated using small scale laboratory test equipment over a range of operating conditions such as pressure, stirring rate (cross-flow rate) and feed composition. Solute rejection/retention, concentration polarisation and hydrodynamic profile are all significant considerations and are not always interlinked (Kawachale et al., 2010). Frontal filtration in laboratory scale cylindrical stirred cells is used extensively for the testing of all types of membrane applications (MF, UF, NF, RO). The relatively simplistic equipment needed and excellent control of the separation conditions make these laboratory stirred cells ideal for practical and experimental purposes, although correct data interpretation requires accurate knowledge of the interfacial events and transport phenomena occurring at the membrane surface (Koutsou and Karabelas, 2012).

Permeate flux decline is an important phenomena inevitably associated with membrane processes. The initial rapid decline is attributed to concentration polarisation (solute build-up) and membrane compaction (deformation of membrane polymer under pressure) while the slower more gradual decline is attributed to physical fouling (microbial adhesion, gel layer formation and solute adhesion) of the membrane surface (Chen et al., 2004). Traditionally the emphasis is on the study of long-term flux decline mechanisms, a topic reviewed by Guo et al., (2012). However, concentration polarisation is noted as a fouling mechanism (Sablani et al., 2001); therefore a fundamental understanding of the dynamics of concentration polarisation can assist in the understanding and prevention of cake formation (Chen et al., 2004) and a decline in process performance (Ma et al., 2008).

Concentration polarisation (CP) is an important factor that influences the performance of membrane separation processes (Kim & Hoek, 2005) mainly by limiting the flux due to increased concentration at the membrane surface (Sherwood et al., 1965). The increased concentration at the membrane surface leads to an equilibrium shift and back diffusion of the permeating solute into the bulk feed solution. This increased surface concentration also leads to a lower flux (solvent transport through the membrane) as a result of the increased osmotic pressure generated. Both phenomena reduce the overall separation efficiency. These phenomena eventually attain a steady state and hence generate a concentration gradient at the membrane surface (Mulder, 1996). The extent of concentration polarisation depends on several factors which are a combination of solute properties, membrane properties and hydrodynamics, namely (Dresner and Johnson, 1980; Nagy et al., 2011):

- competition between solute convection towards the membrane and diffusion away from the membrane.
- the fraction of solute rejected by the membrane.
- the flow regime within the module (whether laminar or turbulent).
- the module geometry.
- the feed velocity tangential to the membrane.
- the properties of the membrane (charge, porosity, permeability)

Characterisation of membrane systems is often performed by studying the rejection of a tracer solute particularly for the determination of membrane MWCO (Rohani et al., 2011). Understanding the mass transfer characteristics of the membrane equipment being used is essential in the accurate determination of membrane rejection properties as the difference between observed and real rejection may be significant. However, once mass transfer is accounted for the real rejection in all equipment will be similar and provides a methodology for scale up between small flat sheet studies to industrial modules.

The objective of this study was to investigate the concentration polarisation and mass transfer effects occurring in three commercially available frontal filtration membrane cells (Amicon, Sterlitech and Membranology) through the analysis of rejection data for poly ethylene glycol (PEG), an uncharged solute.

3.5.1.2 Materials and Methods

The materials and methods used in this section are as described in Section 3.2.1.

3.5.1.3 Results and Discussion

The effect of concentration polarisation is known to increase with increasing membrane flux and dissolved solute concentration (Oatley et al., 2012). Figure 3.8 shows the observed rejection plotted against membrane cell stirrer speed for various pressures for all three cells.

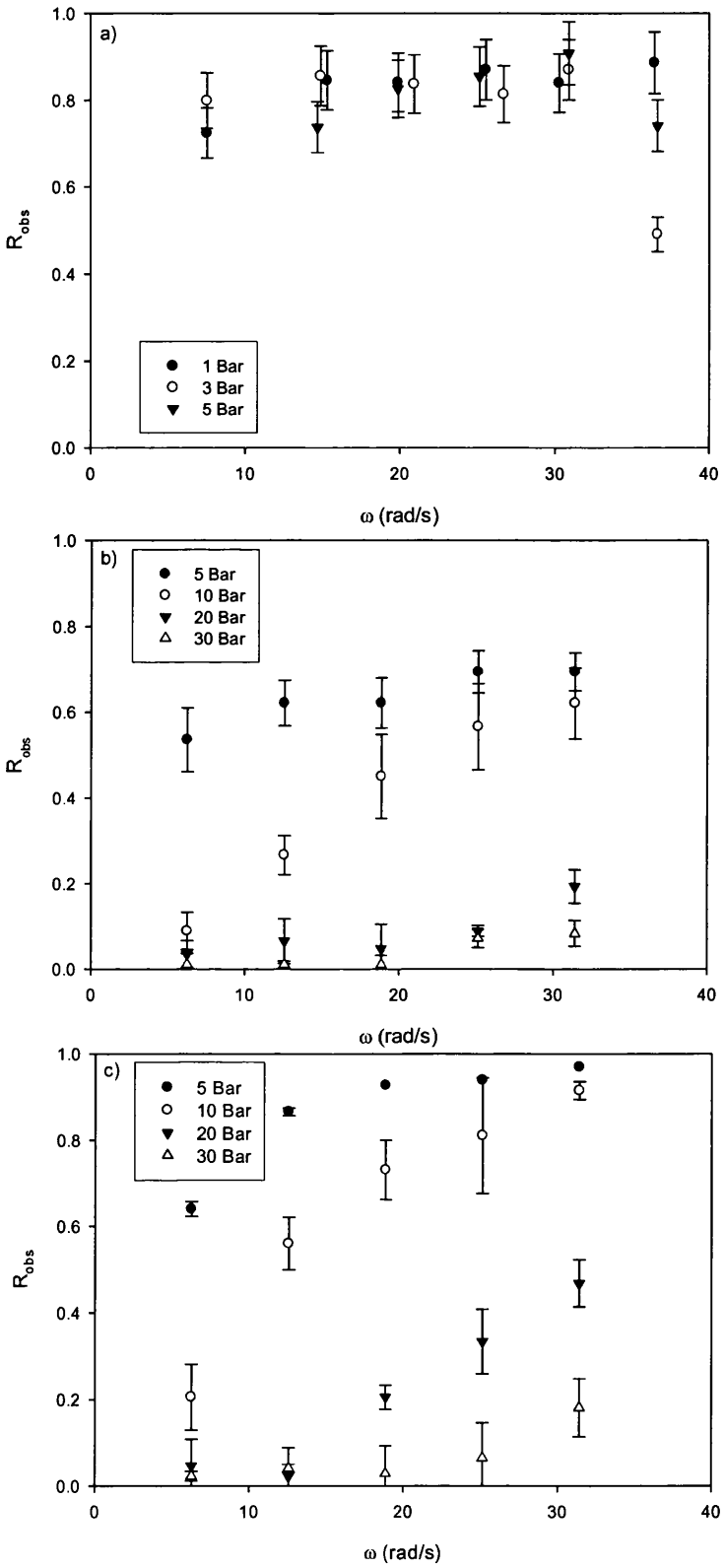

Figure 3.8: Observed rejection versus stirrer speed for a) Amicon cell, b) Sterlitech cell, c) Membranology cell

Each data set shows a distinct variation between the three cells studied. The Amicon cell shows fairly consistent observed rejections of between 80 and 90 %, over the entire range of stirrer speeds, although at the highest stirrer speed (350 rpm) the rejection falls to 50%. Therefore the maximum practical stirrer speed for the Amicon cell was 300 rpm, above this speed the stirrer begins to rotate in a non-uniform manner and the motion is no longer smooth. In addition, the observed rejection for the Amicon cell is seen to be independent of pressure over the experimental range studied. The relationship between rejection and stirred speed shows greater variation in the data for the Sterlitech and Membranology cells when compared to the Amcion cell. Generally, in both the Sterlitech and Membranology cells, observed rejection decreases with increasing pressure indicating the development of a concentration polarisation layer at the membrane surface with increasing flux. This phenomena may be further explained by considering membrane compaction, where the elevated pressures of the Sterlitech and Membranology cells results in the deformation of pores resulting in a slightly decreased rejection. Stirrer speed has a greater effect on rejection in both the Sterlitech and Membranology cells when compared with the Amicon cell, with observed rejection increasing with increasing stirrer speed. The highest observed rejection for the Sterlitech cell was seen at 31.4 rad/s (300 rpm), with a rejection of 62 % at 5 bar, decreasing to 8% at 30 bar. The Membranology cell exhibited a similar relationship as the Sterlitech cell but in this case the maximum rejection was 97% observed at 31.4 rad/s (300 rpm) at 5 bar and a minimum rejection of 18% at 30 bar. The reasoning behind the large differences in observed rejection is attributed in both cases to the build-up of a concentration polarisation layer at the membrane surface as a result of the increased flux associated with increased pressure. However, the Sterlitech cell exhibited a lower observed rejection at similar conditions when compared to the Membranology cell, suggesting that the larger more efficient stirrer in the Membranology cell helps minimize mass transfer effects. Furthermore, the Sterlitech cell stirrer is suspended at a greater height above the membrane surface instantly allowing the wall concentration to be

greater than that of the feed. The stirrer mechanisms are shown in figure 3.9; the three stirrers are suspended above the membrane surface.

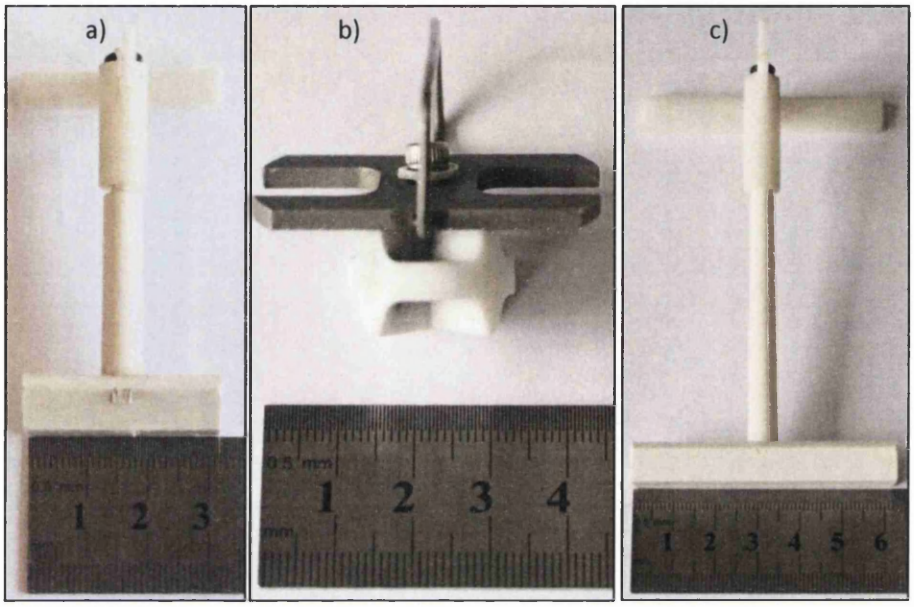

Figure 3.9: Image of the stirrer configuration in the a) Amicon b) Sterlitech and c) Membranology stirred cell

The assembly of the Amicon and Sterlitech cells are documented in the manufacturers' operating manuals (Millipore Corporation 2008; Sterlitech Corporation), whereas the Membranology cell is similar to the Amicon cell. The Amicon and Membranology cells have similar geometry consisting of a sweeping stirrer bar with only a slightly smaller dimension than the width of the stirred cell and is located only 1 to 2 mm above the membrane surface. This 'tight' geometry ensures that the maximum amount of stirring at the membrane surface is available. Furthermore this geometry allows the stirrer to sweep the membrane surface and 'lift' the feed solution from the membrane wall promoting thorough mixing. The Sterlitech cell uses a circular stirrer significantly smaller than that of the cell diameter and sits approximately 6 mm above the membrane surface; therefore, the membrane cell mixing properties are not as favourable as the mixing observed in the Membranology and Amicon cells. The Amicon cell is operated at pressures at or

below 5 bar due to cell material limitations and the resulting maximum flux is much lower than the other two cells. Additionally the effective membrane area is smallest in the Amicon cell closely followed by the Sterlitech cell, with the largest membrane area belonging to the Membranology cell. This explains the fact that the Amicon cell observed rejection is very similar (in the range 70 to 90 %) across the pressure range studied as the flux is generally low (1.726 ml/min at 5 bar, for pure water). Greater variation in observed rejection is noted for both the Sterlitech (1.684 ml/min at 5 bar, pure water) and Membranology (4.154 ml/min at 5 bar, for pure water) cells for similar operating conditions. This is a direct result of the increased flux of the Membranology cell as the membrane area is larger, i.e. there will be higher flux rate for the same applied pressure.

Figure 3.10 displays the observed and calculated real rejection against applied pressure for all three frontal filtration cells studied. Regardless of stirrer speed the general trend shows R_{obs} decreasing with increasing pressure for both the Membranology and Sterlitech cells, however, this effect is not seen in the Amicon cell and this observation is attributed to the low pressure used and the excellent mixing characteristics of the cell resulting in very little concentration polarisation. Note that when the stirrer speed in the Amicon cell exceeds 300 rpm the data becomes anomalous, demonstrating that the stirrer is no longer functioning correctly as previously seen. The real rejection, R_{real}, generally remains constant across each of the cells and is in the region of 80 to 100 %, illustrating that the effects of the stirrer speed have now been removed. The scatter of the data is more evident with the Amicon and Sterlitech cells, with the Membranology cell providing almost consistent data at every stirrer speed and pressure. This could be attributed to the fact that the Membranology cell is the largest of the three cells studied and thus the data collected is more consistent due to reduced experimental error. The real rejection for the Amicon cell is very comparable to the observed rejection, with a greatest difference of 9.4% observed at 5 Bar and 360 rpm (a stirrer speed which has previously been noted as unreliable). The average difference between observed and real rejection for the Amicon cell was 0.2 %, i.e. the values are the same if one

accounts for experimental error. This suggests that the is very little, if any, concentration polarisation occurring in the Amicon cell and the mass transfer effect is negligible. This result is similar to a previous study by Bowen et al. (2004) who reported a difference of 3.7 % between real and observed rejection in an Amicon 8400 (400 mL capacity) stirred cell. The real rejection for the Sterlitech and Membranology cells differ greatly when compared with the observed rejection and the average difference between the observed and real rejection is 68.4 and 45.6 % respectively. This indicates that significant concentration polarisation is occurring in each of these cells.

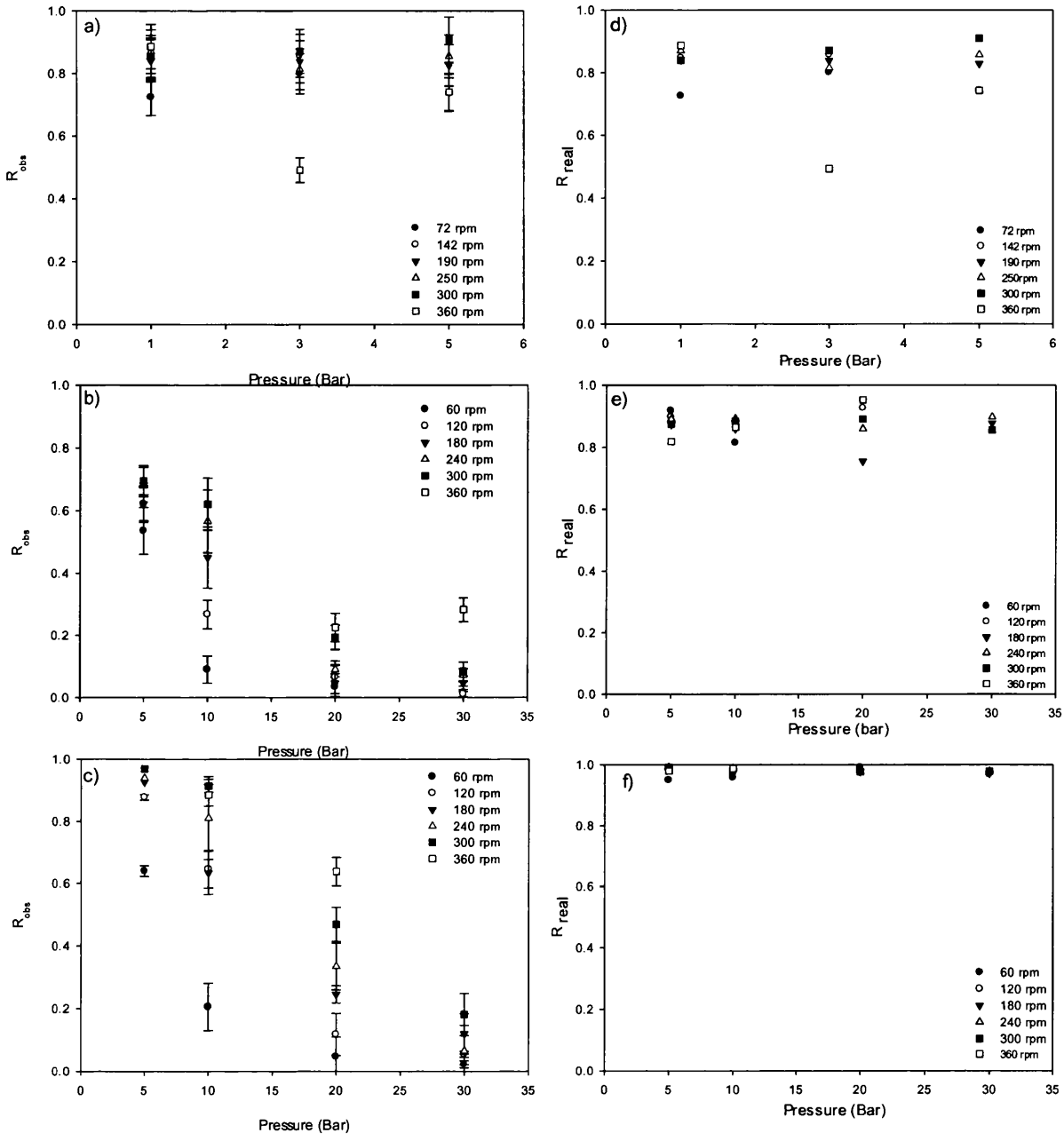

Figure 3.10: Observed rejection versus pressure for a) Amicon cell, b) Sterlitech cell, c) Membranology cell and real rejection versus pressure for d) Amicon cell, e) Sterlitech cell, f) Membranology cell

The greatest difference between observed and real rejection was at the highest pressures with lowest stirrer speeds as would be expected. Previous experimental work carried out by Nguyen et al. (1980) and Bowen et al. (1997) used maximum

applied pressures of 1.2 and 5 bar respectively. In these two studies, and similarly for the Amicon cell in this study, there was very little mass transfer occurring. However, this study with the Sterlitech and Membranology cells show that as pressure increases, the flux rate increases and the resulting mass transfer is governed by convective transport of solute to the membrane surface. This convective flux to the membrane surface is significantly higher than the mixing rate removing solute from the membrane surface and thus concentration polarisation is inevitable. Thus, if a membranologist was to conduct feasibility studies in each of these three cells for potential new applications, then the results obtained would show an unfavourable process if the Sterlitech or Membranology cell was used without a knowledge of this mass transfer effect. This is clearly illustrated by considering the case of 60 rpm at 30 bar for the Membranology cell where the observed rejection is < 5 %, i.e. no feasible separation process, and the real rejection is > 95 %, which would be considered to be an excellent separation.

An experimental observation made for all three cells is that as the stirrer speed increases (especially as the feed level decreases) vortexing is induced within the cells. When the vortexing is significant, the cone can extend from the top of the feed liquid to the membrane surface creating a smaller observed membrane active area. In this study this effect was reduced as much as possible by operating at the maximum cell volume. Furthermore, concentration polarisation and mass transfer descriptions are inaccurate when significant vortexing occurs. The experimental work undertaken in this thesis would suggest that the optimum stirrer speed to use in the Amicon cell would be 300 rpm. A previous study that developed mass transfer correlations for the Amicon cell has reported using stirrer speeds up to 1300 rpm. This high stirrer speed could not be obtained in this study using similar equipment (the stirrer simply would not turn in the magnetic field) and with the observation of vortexing this would question the validity of the data produced. A general recommendation from this work would be to investigate improved mixing strategies to avoid vortexing in frontal filtration cells which should reduce concentration polarisation and minimise fouling.

The mass transfer coefficient was calculated using the infinite rejection method, see Eq [3.10]. The three data sets obtained as Figure 3.10 were further manipulated and plotted in the form $ln\ [(1 - R_{obs})/R_{obs}]$ against J_w/ω^n and are shown in Figure 3.11.

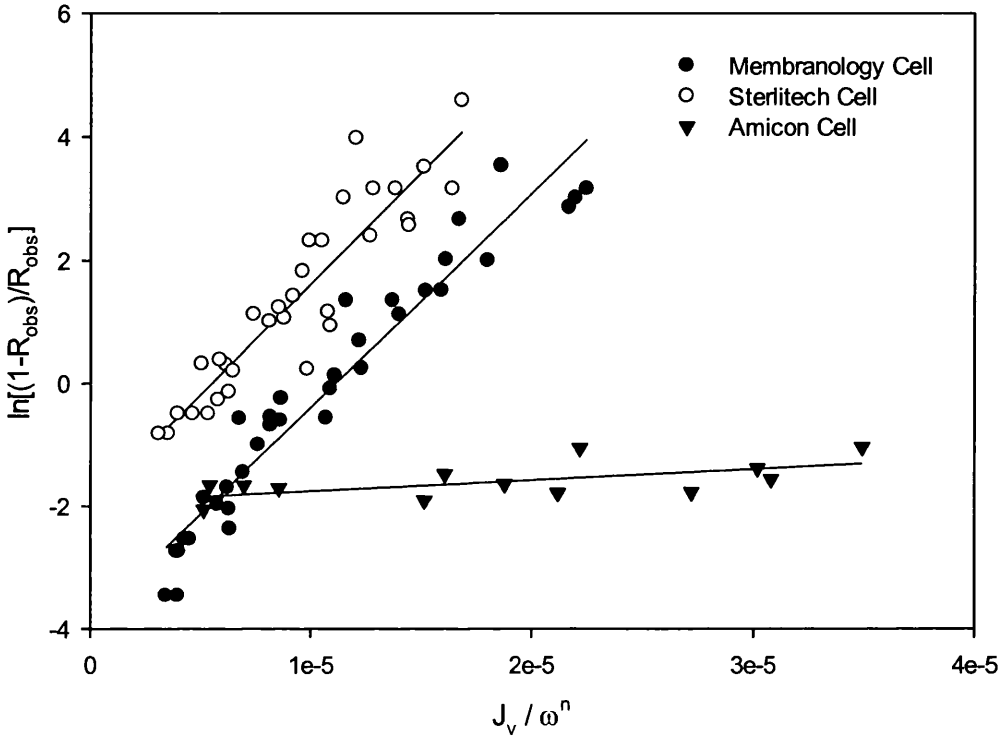

Figure 3.11: Linear analysis to determine the mass transfer coefficients of the Membranology, Sterlitech and Amicon membrane cells

The value for the exponent *n* for each cell was determined by optimisation of the coefficient of determination (R^2). The *n* and *a* values obtained from the experimental data are shown in Table 3.2.

Table 3.2: Mass transfer coefficients of the three membrane cells studied

	a	n
Amicon	1.080E-05	0.567
Sterlitech	2.771E-06	0.487
Membranology	2.993E-06	0.415

Figure 3.11 facilitates the comparison of the mass transfer effect within each of the three cells by comparison of the gradients of the best fit line, the greater the gradient the higher the mass transfer effect. The Amicon cell displayed a shallow positive gradient and therefore confirms that the Amicon cell has very little mass transfer occurring. The Sterlitech and Membranology cells display a gradient of approximately 45°, suggesting the difference between observed and real rejection is increasing as the J_v/ω^n term increases. As the gradient is similar for both cells, this indicates that the mass transfer effect is also similar in both cells and much greater than that of the Amicon cell. However, the flux rate obtained in the Membranology cell was far superior when compared to the Sterlitech cell due to the larger membrane area. This indicates that the true mass transfer effect is less in the Membranology cell when compared to the Sterlitech cell as a result of the better mixing rates generated by the superior stirrer design. The large range of extrapolated values of R on the y-axis can be explained by the varying cell observed rejections. The observed rejection versus pressures for the Sterlitech and Membranology cells display an observed rejection ranging from 10 to 90% which explains the large data spread in Figure 3.11 for the Sterlitech and Membranology cells.

The calculated mass transfer coefficient (Eq. [3.5]) from this study was compared to the mass transfer coefficient obtained from the correlation (Eq. [3.9]) first proposed by Nakao and Kimura (1981) and used by Bowen et al. (1997) using the updated mass transfer constants derived in this study (Table 3.2) and is shown in Figure 3.12. The correlation greatly under exaggerated the mass transfer effect within the cells when compared with the experimental data obtained in this study. For

example, the Amicon data (Figure 3.12a) gives an experimental mass transfer coefficient of ~$3.4x10^{-5}$ m s^{-1} which corresponds to a theoretical value of ~$2.8x10^{-6}$ m s^{-1}, which is a significant underestimation. The correlation in question was developed for UF membranes, however, the membrane used in this study is representative of a very tight UF or loose NF and this may explain the discrepancy observed.

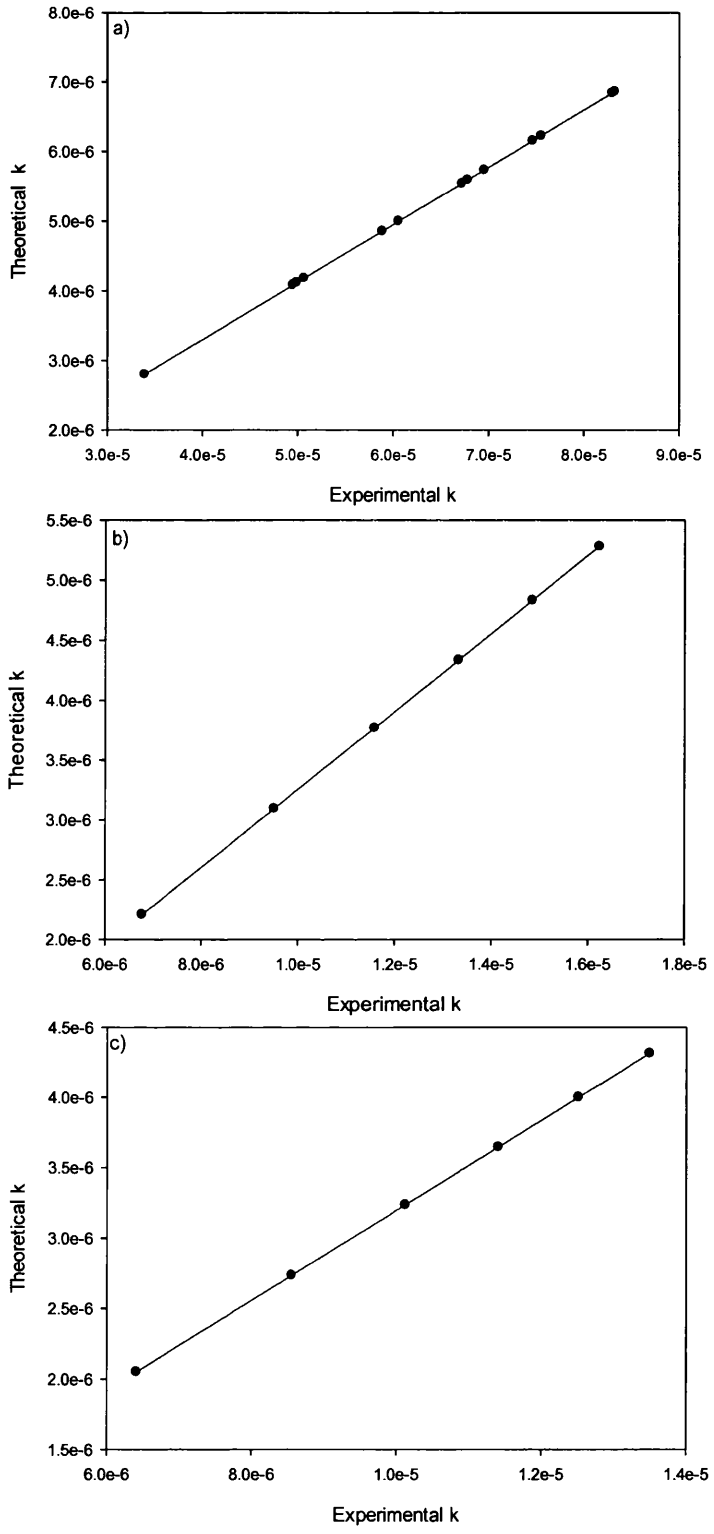

Figure 3.12: Comparison between experimental and theoretical mass transfer coefficient for the a) Amicon b) Sterlitech and c) Membranology stirred cell (Error bars omitted as error is low (<1%))

The polarisation modulus was calculated from the experimental data and is shown in Figure 3.13.

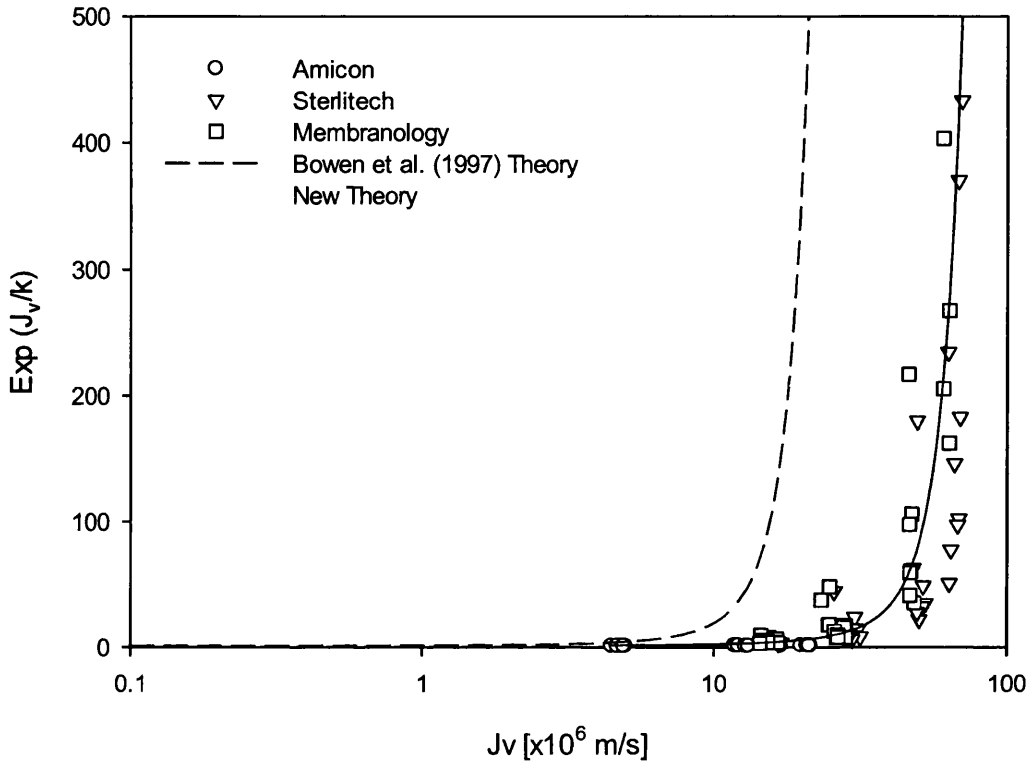

Figure 3.13: Comparison of calculated mass transfer effect to that of new proposed theory

The previous correlation (Bowen et al., 1997) is plotted as a reference guide and the Amicon data is seen to lie just outside of the correlation (abscissa region 1 to 10) as expected. However, in this case, the rest of the data points also deviate significantly from the correlation data. This is potentially due to the fact that the flux is an order of magnitude greater than the mass transfer coefficient and the resulting polarisation modulus is very large. For example, at a flux of 63 $x10^{-6}$ m s^{-1} and 300 rpm for the Sterlitech and Membranology cells, the correlation predicted a polarisation modulus of $6.4x10^{5}$ and $8.3x10^{8}$ respectively. These two values are ridiculously large and the experimental data obtained for the Sterlitech and Membranology cells respectively gave a polarisation modulus of 77.5 and 162.6 at similar conditions. The theoretical data obtained for the Amicon cell was larger than that of the experimental (theoretical polarisation modulus of 31.4 at a flux of

21 x10^{-6} m s^{-1} at 300 rpm and a experimental polarisation modulus of 1.3 for the same conditions), however, this difference was insignificant when compared to the error in the Sterlitech and Membranology cells. As stated previously, the theoretical correlations relate to UF membranes where the pressure applied is small. However, for NF membranes the pressure used is much higher to obtain high flux rates. The data in Figure 3.13 would suggest that the concentration polarisation follows similar behaviour to that of UF membranes but is off-set by an order of magnitude. This is a similar observation to that seen previously using the NF270 membrane in cross flow mode (Oatley et al., 2012). To account for this off-set, a dimensionless term was added to Eq. [3.7] and is given as:

$$\left(1 + \left(\frac{J_v}{\omega r}\right)^x\right) \qquad [3.12]$$

where *x* is an empirical coefficient. Therefore, manipulating Eq. [3.7], the equation used to predict the mass transfer effect now becomes:

$$N_{Sh} = \varphi(N_{Re})^n(N_{Sc})^{0.33}\left(1 + \left(\frac{J_v}{\omega r}\right)^x\right) \qquad [3.13]$$

The additional term will account for the off-set in the polarisation modulus seen at high flux rates for this membrane and when the flux rate is low then the additional term will become negligible and the correlation will revert back to Eq. [3.7]. Introduction of this correction factor resulted in the mass transfer coefficient for each of the cells being calculated as a much higher value and the resulting polarisation modulus being more comparable to that observed experimentally, as can be seen in Figure 4.1.6.

3.5.1.4 Conclusion

A mass transfer study has been conducted using three commercially available laboratory frontal filtration membrane cells using PEG 3400 and a 4000 MWCO membrane. The Amicon cell was used as this equipment has been widely

implemented for laboratory UF membrane studies. However, the cell is largely impractical for NF applications due to the 5 bar maximum operating pressure and should be considered only suitable for MF and UF applications. Both the Sterlitech and Membranology cells used in this study are high pressure cells and are suitable for NF applications. This work has demonstrated that a maximum stirrer speed of approximately 300 rpm is suitable for most applications using each of the membrane cells studied. Concentration polarisation was not observed, or was very minimal, when using the Amicon cell and this was attributed to the low flux rates obtained as a result of the low pressure operation. However, the increased flux rates generated in the Sterlitech and Membranology cells as a result of the increased operating pressures developed a significant concentration polarisation layer leading to disparity between observed and real rejection (a very important concept when considering process scale up). The mass transfer effect observed in the high pressure cells was similar, however, due to the Membranology cell having a larger membrane area the cell showed better flux performance and was superior when compared to the Sterlitech cell, i.e. the same mass transfer effect at an increased flux rate.

The experimental mass transfer results obtained were compared to the generally accepted theoretical correlation and the correlation was found to significantly under-estimate the magnitude of the mass transfer coefficient for the equipment used. The theory was then modified to include a correction factor to account for the high flux and larger mass transfer effect. This correction factor facilitated a more accurate prediction of the polarisation modulus and was accepted as a reasonable description of the interfacial phenomena occurring. This work suggests that this new theoretical mass transfer correlation is more appropriate for NF and tight UF membranes in frontal filtration mode. However, this work does show that blindly using mass transfer correlations developed for UF applications can lead to significant errors when considering NF applications. More work is required with different NF membranes to further support this model.

Having successfully determined the mass transfer effects within these three commercially available frontal filtration cells, the prudent scientist or engineer can now use this information for increased process understanding during the evaluation and scale-up of new membrane processes.

Appendix A2: Characterisation of the NTR-7450 Nanofiltration Membrane

3.5.2.1 Introduction

In order to achieve a successful implementation of a membrane separation process having a defining value of the membranes separation characteristics to at least provide a starting point in terms of membrane selection is very useful. In the case of nanofiltration this information is normally reported as the molecular weight cut-off (MWCO) or nominal salt rejection value for a mono-valent (mostly NaCl) or di-valent salt. MWCO is a correlation of rejection against an uncharged solutes molecular weight. The molecular weight cut-off is normally reported as the molecular weight of a solute, at which 90% is rejected (Van der Bruggen and Vandecasteele, 2002). However, there is no standardisation of this 90% value (Mehta and Zydney, 2005; Rohani et al. 2011), with different manufactures using solutes with different physical properties, different concentrations and different operating conditions, resulting in membranes that may have the same reported properties but with very different characteristics when applied to the same separation. The use of nanofiltration for desalination often sees properties of membranes quoted as a percentage of salt rejection, often quoting a value for both a mono-valent and di-valent salt. However, similar to a reported MWCO, the values obtained are dependent of the salt concentrations and operating conditions used. The salt concentrations used to characterise a membrane is particularly important as rejection can significantly decrease with increasing salt concentration.

These factors result in a significant disparity between characterisation results obtained through academic studies and membrane manufacturers, resulting in confusion for the end-user trying to implement a novel membrane process. End-users do not only have to contend with different information from different manufacturers (who use different characterisation methods) but also differences in polymeric membrane materials. Overall, the provision of a MWCO or a salt rejection value should, in theory, provide suitable information to allow for the proper engineering of a membrane system in order to design a process that has predictable performance. The standardisation of a characterisation method should allow for simpler identification of a suitable membrane for a required process,

reducing the number of optimisation steps required when evaluating the membranes in a specific system.

Membrane performance (flux and rejection) is affected by three major solute-membrane interactions: steric exclusion (sieving effect), Donnan (charge) interactions, and solute-membrane interactions (i.e., dielectric effects, hydrogen bonding, hydrophobic attraction, etc.).

This subsection aims to characterise the NTR-7450 membrane and subsequently compare the values obtained to those values reported in the literature. The NTR-7450 membrane was selected for the characterisation as the membrane was used for the separation of fungal bioactives in chapter 5 and for the separation of potato bioactives in chapter 6.

3.5.2.2 Characterisation Methods

In this study Polyethylene glycol (PEG) have been used to perform the membrane MWCO characterisation experiments. PEGs are suitable for MWCO characterisation experiments since they are cheap and available in bulk and therefore are accessible to all concerned with calculating MWCO. PEGs are available in a wide range of molecular weights, are easily analysed by a variety of methods and furthermore exhibit low solute membrane interaction, resulting in insignificant irreversible solute adsorption to the membrane surface (Rohani et al. 2011).

The use of a MWCO to describe membrane separation is further complicated by the emergence of organic solvent nanofiltration (OSN). The determination of a MWCO in an organic solvent system is much more difficult due to the effects of process conditions, inherent properties of membranes and the solvent-solute-membrane interactions (Zwijnenberg et al., 2012). The determination of a MWCO is complex in OSN due to the profound effect a solvent may exhibit on a membrane polymer, where polymer swelling may be observed, therefore altering the membrane properties dependent on solvent used (See-Toh et al., 2007). This would suggest that any MWCO characterisation reported requires not only information on the solute and operating conditions but also the solvent used.

3.5.2.3 NTR 7450 membrane properties

The NTR 7450 membrane is a widely used sulphonated polyethersulphone thin film composite nanofiltration membrane produced by Hydranautics (Nitto Group, California, USA). The NTR 7400 series were first developed in the late 1980s by the Nitto Electric Industrial Company, Japan as an alternative to the earlier NF membranes who despite exhibiting high water flux and salt rejections suffered poor chemical resistance (less that 1 ppm chlorine)(Ikeda et al., 1988). The NTR 7400 series was designed to withstand up to 10 ppm chlorine allowing for much harsher cleaning cycles to be used and hence reduce fouling and maximise membrane life. Similarly to the previous NTR 7250 the NTR 7400 series when developed were shown to exhibit high water flux, while displaying resistance to high and low pHs as well as temperature resistance. The original characterisation of the NTR 7450 membrane by Ikeda et al. (1988) suggested the membrane to be stable when stored in 10,000 ppm chlorine for a one month period. Interestingly for the NTR 7450 membrane, salts containing monovalent cations have higher rejections than salts containing divalent cations, a reverse of what is normally expected in charged nanofiltration membranes. This phenomenon is due to the negative charge of the sulfonated polyethersulfone active membrane layer (Figure 3.14), resulting in higher rejections of negatively charged solutes when compared with neutral solutes of similar size.

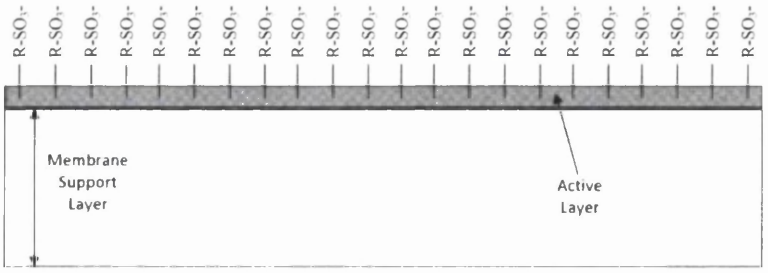

Figure 3.14: Diagram of NTR 7450 membrane sulfonated active layer

The NTR 7450 membrane has found many applications in a wide range of sectors including treatment of electroplating wastewater (Wang et al., 2007), dye removal (Van der Bruggen et al., 2001), arsenic removal (Ahmed et al., 2010), paper effluent treatment (Nystrom et al., 1995), groundwater treatment (Her et al., 2008),

desalination (Bargeman et al., 2014) and waste water treatment (Liikanen et al., 2003; Kaufman et al., 2014) among others. Furthermore, the NTR 7450 membrane has been extensively characterised by a number of academic studies (Bowen and Mohammad, 1998; Van der Bruggen et al., 1999; Van der Bruggen and Vandecasteele, 2002; Bargeman et al., 2005; Boussu et al., 2005; Mi et al., 2006, Coronell et al., 2010; Shirley et al., 2014), however, the results reported for the membrane are highly variable.

The wide range of reported properties of the NTR 7450 membrane make prediction of membrane separation characteristics almost impossible, with the MWCO as an example being reported to range from 200 – 1000 Da (Wang et al., 2007; Bargeman et al., 2005). These MWCO values would place the membrane in the range of tight NF to very loose NF or even a tight UF membrane. Interestingly, the MWCO values reported in the literature (Table 3.3) for the NTR-7450 membrane are highly varied with no discernible pattern linking the MWCO and the characterisation method used. The MWCO reported by Shirley et al. (2014) varied from 210 to 870 dependent on the concentration of solute used for the characterisation. The study undertaken found that the MWCO decreased from 870 Da to 210 Da when the solute concentration increased from 0.02 mmol/l to 3 mmol/l indicating a membranes MWCO is highly dependent on solute concentration used. Furthermore, a large solute with a large dipole moment caused an increase in solute rejection. The largest value for the membrane MWCO was reported by Her et al. (2008) with a value of 1640 Da, obtained by using PEGs as a characterisation method. Interestingly, Schaep et al. (2001) used mixed PEGs and a MWCO of 310 Da was reported. The value of 1640 obtained by Her et al. (2008) was obtained using a range of polyethylene glycols from 200 to 10 000 Da. The initial results obtained by the study failed to reach 90% rejection even with PEG 10 000, therefore the concept of effective pore diameter was used reaching a final MWCO of 1640 Da. The wide range of data reported in the literature may be attributed to the characterisation methods. The solute concentration as shown by Shirley et al. (2014) is significant in determining the MWCO which would help partially explain the large range of MWCOs reported. Furthermore, the membrane pre-treatment

method (pre-compaction) used (not always reported in the literature) and the pressures at which the characterisation experiments are undertaken may also affect the membrane MWCO.

Table 3.3: Measured NTR-7450 membrane MWCO reported in literature

MWCO	Characterisation Method	Reference
210 – 870	Mixed Solutes	Shirley et al. (2014)
310	Mixed PEGs	Boussu et al., (2005)
600 – 800	Mixed Sugars	Schaep et al., (2001)
600 -800	Mixed Organic Solutes	Van der Bruggen et al., (1999)
1000	-	Manufacturer (Bargeman et al., 2005)
1075	Mixed PEGs	Rohani et al. (2011)
1640	Mixed PEGs	Her et al. (2008)

The membrane MWCO is a parameter that reports the performance of a membrane to an uncharged separation. The quoting of the membrane MWCO may not always be favourable as the value is highly dependent on the characterisation method used, therefore the pore size (often pore radius) is also reported. Pore size is often characterised in a number of ways, using technology such as Atomic Force Microscopy (AFM), BET (Gas adsorption), liquid-liquid displacement, and neutral solute rejection. However, similarly to the use of an uncharged solute to calculate MWCO the measurement techniques for pore size often report a slight variation in the values. This variation observed is somewhat expected due to the very small pore radii present in a nanofiltration membranes, a value of approximately 1nm and the resolution limits of the characterisation techniques.

The pore size values found in the literature are varied for the NTR 7450 membrane. Bowen and Mohammad (1998) reported a pore radius of 1.41 nm obtained through the use of the Hagen-Poiseuille equation related to membrane permeability, whereas Wang et al. (1995) reported a pore radius of 0.7 nm obtained by using the steric pore hindrance model. Bargeman et al. (2005) used uncharged solutes

(glucose) to characterise a number of nanofiltration membranes and reported a pore radius of 1.34 nm for the NTR 7450 membrane, however, in a later paper Bargeman et al. (2014) estimated the pore radius to be 0.62 nm again using glucose as the characterisation solute. Furthermore, Schaep et al. (1998) used neutral solutes for estimation of the NTR-7450 pore radius obtaining a value of 0.8 nm.

The pure water flux is an often quoted performance parameter of a membrane. An ideal membrane would exhibit high solute rejection/permeation (efficiency of separation) at a high flux rate (rate of separation). The NTR-7450 membrane pure water flux values obtained from the literature are shown in Table 3.4. The values range from 2.00 LMH/bar to almost 17.00 LMH/bar which represents a significant difference of 15 LMH/bar. The average flux of the data collected from the literature is 9.90 LMH/bar. The range of values reported may be attributed may again be attributed to the membrane pre-treatment methods used, the configuration of the membrane (frontal or cross-flow modes) or the representativeness of small laboratory scale membrane sheets used (i.e. variation in membrane manufacturing process).

Table 3.4: NTR-7450 membrane permeance values reported in literature

Pure Water Permeance (LMH/bar)	Reference
2.00	Wang et al., (2007)
6.30	Bargeman et al., (2014)
7.50	Bargeman et al., (2005)
9.17	Ikeda et al. (1988)
9.18	Bowen and Mohammad (1998)
9.20	Nystrom et al. (1995)
10.71	Rohani et al. (2011)
11.90	Manttari et al., (2000)
12.90	Van der Bruggen et al., (2001)
13.00	Manttari et al. (2006)
16.99	Van der Bruggen and Vandecasteele (2002)

The contact angle of a surface against water represents the surfaces wet-tability. When a droplet of water wets the membrane surface, the interaction between the membrane and the droplet represents the contact angle. For instance a small contact angle shows the membrane surface has the ability to interact with the water molecules (dipole interaction). The membrane material and for example the presence of dissociable groups on the membrane surface increases the interaction with the water molecules and makes the surface more hydrophilic. Interestingly, the contact values reported in the literature for the NTR-7450 membrane are 52°, 58° and 69.6° by Her et al. (2008), Manttari et al. (2006) and Boussu et al. (2005) respectively, all of which suggest a highly hydrophobic membrane. For comparison the contact angle of the NF270 membrane is 30° suggesting a rather hydrophilic membrane (Manttari et al., 2004). Polyethersulphone is a fairly hydrophobic material, and the addition of the sulphonic acid groups should make the membrane more hydrophilic. The presence of sulphonic acid groups in the membrane surface

material should allow for interaction between the surface and the water molecules, however, the large contact angle values suggest that the sulphonic acid groups are missing from the membrane active layer (Manttari et al., 2006).

Nanofiltration membranes are often used as a pre-treatment method for reverse osmosis desalination systems. The use of a nanofiltration membrane reduces the loading onto the reverse osmosis by often removing high percentages of divalent salts and some of the monovalent (mainly NaCl) present in the feed. The salt removal properties of the NTR-7450 membrane has been widely studied, however like many other membrane properties the separation properties are dependent on the feed properties. The NaCl rejection value is often quoted as 50% for the NTR-7450, however Schaep et al. (1998) reported the retention decreasing from over 50% at a concentration of 0.05 eq/l to less than 10% at a concentration of 0.75 eq/l at 10 bar. Nystrom et al. (1995) reported a NaCl rejection of 51% at a concentration of 0.5 g/l and at 10 bar for the NTR 7450 membrane. The rejection of $MgCl_2$ is reported as 13% lower than that of NaCl at similar conditions, while Na_2SO_4 and $MgSO_4$ have rejections of 92 and 32 % respectively (Ikeda et al., 1988). These values suggest that the NTR-7450 membrane exhibits classical Donnan exclusion behaviour for a negatively charged membrane.

The NTR-7450 membrane properties reported in the literature provides a great deal of confusion to end users attempting to implement a membrane based process. It is worth noting that the NTR-7450 is only one example of the confusion that surrounds membrane properties. The lack of a standardised method for membrane characterisation makes the selection of a membrane for a certain application almost impossible without laboratory trials, particularly at the nano scale where a number of complex phenomena are taking place simultaneously. The NTR-7450 membrane characterisation reported in this work attempts to offer further insight into the membrane properties of the membrane without adding further confusion to the situation.

3.5.2.3 Results and Discussion

Electrokinetic Study

The results of the membrane electrokinetic study are shown in Figure 3.15. The magnitude of the zeta potential is proportional to concentration, with lower zeta potential values observed at higher salt concentrations. The zeta potential curves obtained for the NTR-7450 membranes are typical for strongly acidic materials (Möckel et al., 1998). The results obtained suggest an iso-electric point at pH 3, however, the presence of an iso-electic point is in reality unlikely as the quantity of HCl acid required in order to achieve a pH of 3 results in the equipment detecting H^+ ions from the acid. Many researchers use a minimum pH at which the number of the H^+ ions in solution does not exceed more than 10% of the initial ionic strength (i.e. pH_{min} = 4 for $1x10^{-3}$M) (Szymczyk et al., 1998). Therefore the values at which an iso-electric point are observed exceed the suggested 10% limit. Overall the membrane is at an almost constant negative charge over the whole range of pH studied, displaying no iso-electric point over the pH range 4 - 10.

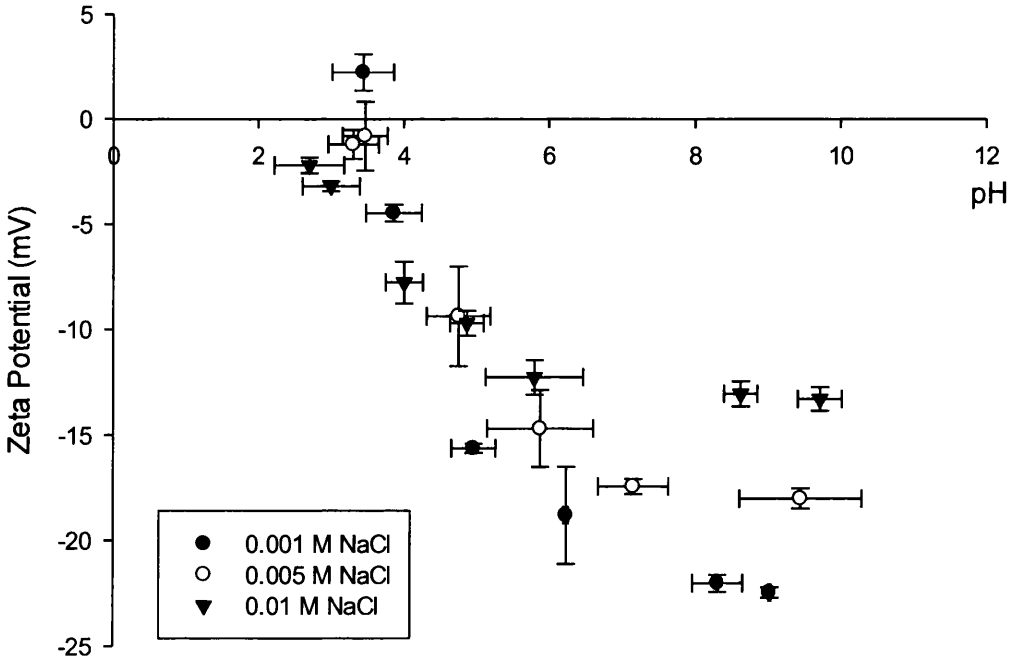

Figure 3.15: ζ-potential measurements at various NaCl concentrations and pH

Membrane Flux

Membranes are often pre-compacted prior to any experimental work in order to reduce any compaction effects (Zwijnenberg et al., 2012; Bargeman et al., 2014), although some research negates this compaction stage. Membrane pre-conditioning serves several purposes, such as removing any conditioning/preservative agents that may be present following manufacture. Furthermore the pre-compaction stage ensures that the membrane has reached steady state where the membrane compaction, swelling and solvation has reached equilibrium and as such the membrane performance is at a steady state. The pre-compaction of membranes minimises the compaction effects that occur with operating pressures which in turn affects the membrane separation properties. Often, membrane researchers compact the membrane at the maximum operating pressure for a nominal time period (rounded time figures such as 1 hour)(Hilal et al., 2005), however this selection of such a time basis contains no scientific basis, therefore the time required for compaction of the NTR-7450 membrane was investigated. The membranes were cut, rinsed and stored overnight in ultrapure water in order to remove any deposits or preservative chemicals present prior to experimental work. The compaction was undertaken at a pressure of 30 bar and the flux is displayed in Figure 3.16.

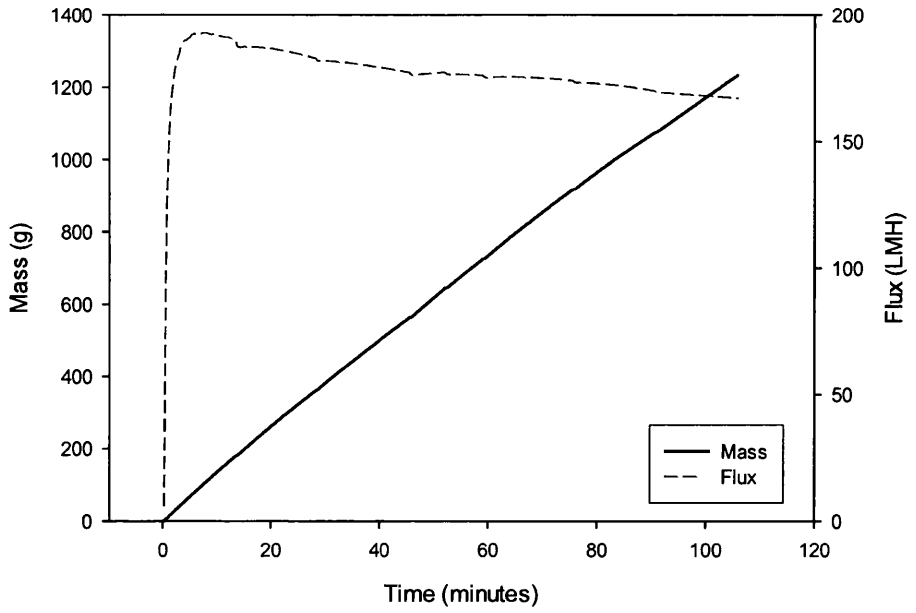

Figure 3.16: NTR-7450 pure water flux during pre-compaction at 30 bar (Single run results displayed)

The initial pure water flux as shown in Figure 3.16 reached a value of 190 LMH however the flux continued to decrease over the time period studied reaching a final flux of 167 LMH after a period of 105 minutes. The final flux was reached and maintained for a period of 10 minutes therefore was treated as the final flux value i.e. the membrane had reached a constant flux. The results obtained suggest that selecting a nominal time period in order to compact the membrane may lead to incorrect results due to further compaction during processing. Therefore membrane flux should be carefully monitored during preconditioning with a pure solvent until a constant flux is reached and maintained. Due to the results obtained the membranes were compacted for a period of 2 hours prior to any characterisation experiments.

The pure water flux is an excellent membrane comparison parameter which allows the evaluation of membrane separation rates between different membranes with similar MWCO. The pure water flux value obtained for the NTR-7450 membrane was 3.2 LMH/bar (Figure 3.17) a value which is at the lower end of those reported in Table 3.1.1. Also worth noting is that the highest flux values obtained at 30 bar

was ~110 LMH, a much lower value than that obtained as a final flux during the membrane compaction (a value of 5.5 LMH/bar). Interestingly when the membrane 'relaxation' was studied the results suggested that once compacted the membrane pure water flux was reproducible as shown in Figure 3.17 (0 hours is the initial pure water flux, 3 hours and 16 hours is the pure water flux when the membrane has been left unpressurised for 3 and 16 hours respectively). The pure water flux values were obtained using a different membrane to that used to determine the compaction effects suggesting an inherent variation between each membrane studied. This phenomena may be an inherent issue when studying membranes on a laboratory scale. The use of industrial scale membrane modules may reduce the variation observed due to slight variations in the manufacturing process, i.e. average any variations over a larger membrane area. In addition to pure water flux, the effect of membrane relaxation was studied, discovering that no relaxation of the polymer structure is observed once the membrane is initially compacted. This irreversible deformation of the membrane suggests that the membrane experiences plastic deformation as opposed elastic deformation.

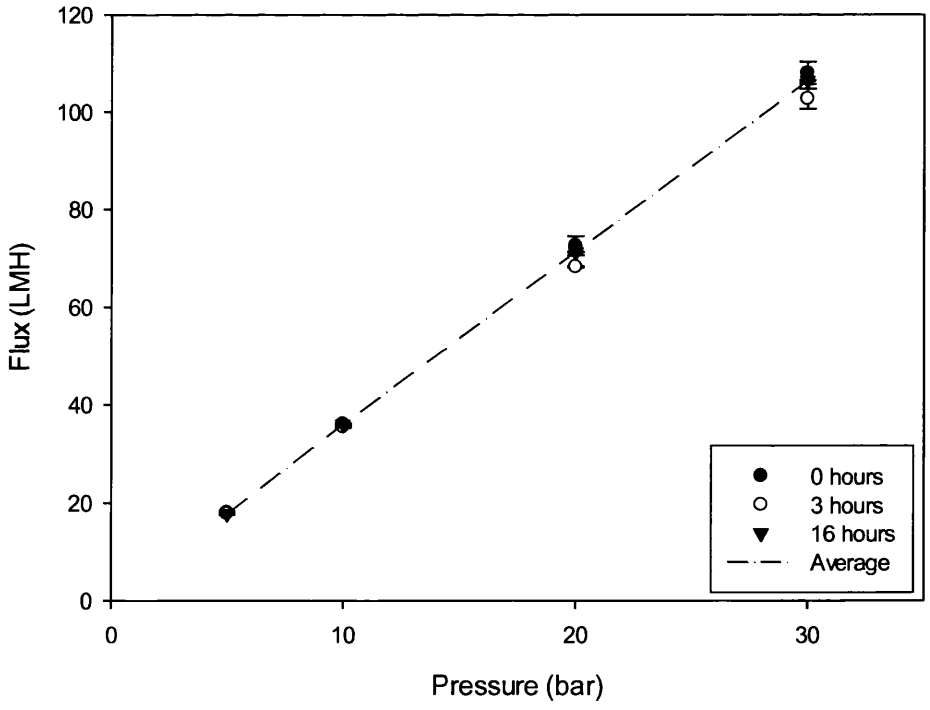

Figure 3.17: NTR-7450 pure water flux at various pressures

PEG Rejection Experiments

In order to calculate the membrane MWCO a selection varying MW PEGs were used spanning the range on MWCOs reported in Table 3.3. Single PEG solutions were studied in this experimental work in order to obtain well defined rejection values using the selected analysis method. The observed and real PEG rejections versus pressure are displayed in Figures 3.18 and 3.19 respectively.

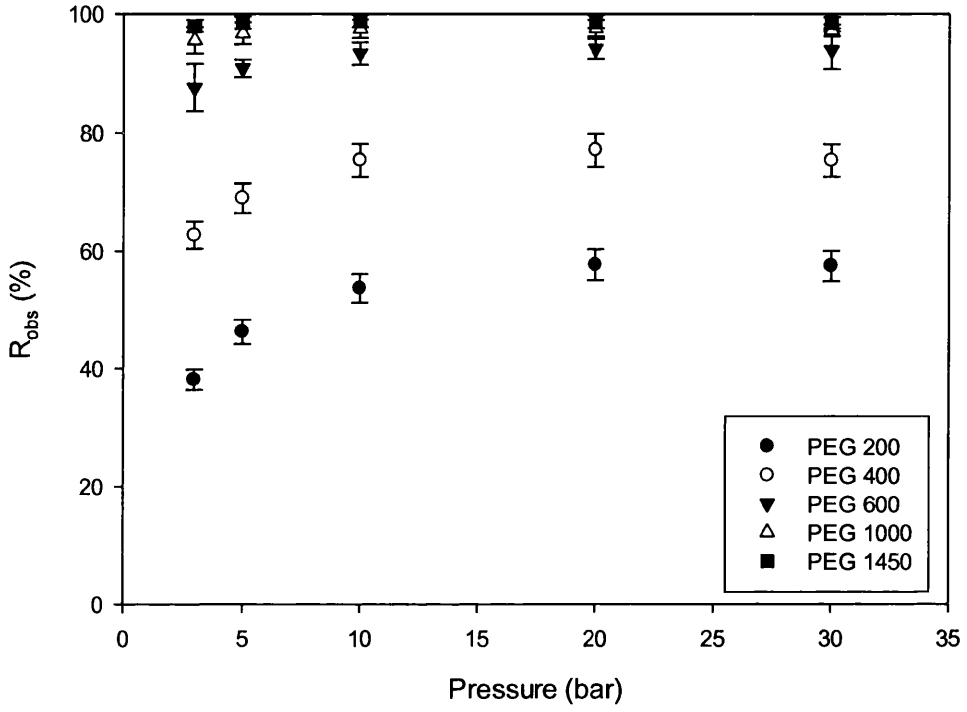

Figure 3.18: PEG observed rejection versus pressure

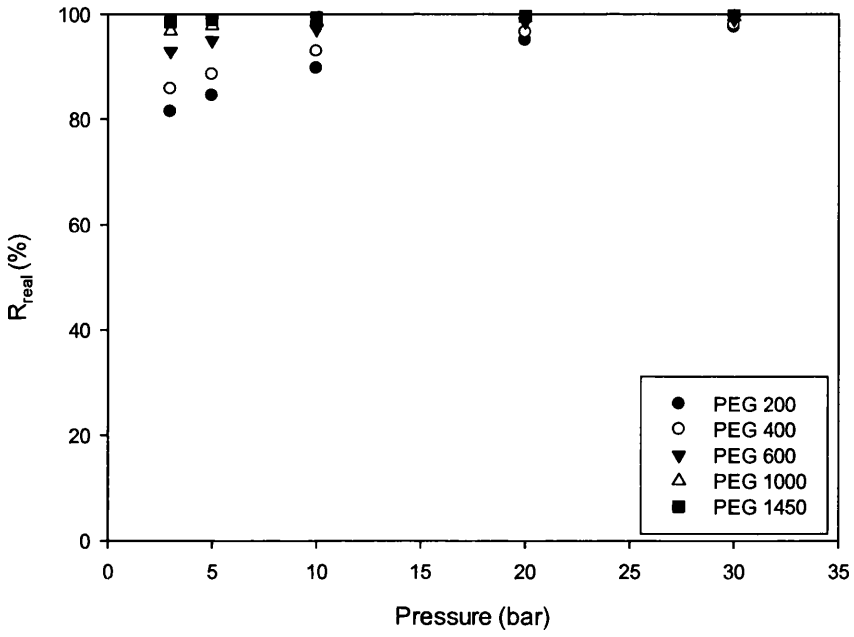

Figure 3.19: PEG real rejection versus pressure

The observed rejection versus pressure shows that as the pressure increases the rejection increases for each of the PEGs studied. The limiting rejection is reached at approximately 15 bar for all the PEGs studied, with some decrease in observed rejection observed at 30 bar as expected due to mass transfer effects. In all cases the maximum rejection of each solute studied was obtained at 20 bar. The rejection increases with increasing molecular weight, with PEG 200 exhibiting the lowest observed rejection while PEG 1450 displayed the highest observed rejection with values of 58 and 98 % respectively at 20 bar. The correction for mass transfer using the infinite stirrer method (as described in section 3.4) results in the rejection values increasing. The lowest real rejection values were still obtained at the lowest pressure (5 bar) studied, however, the real rejection for PEG 200 was approximately 82% as compared to an observed rejection of approximately 38%.

In order to calculate the membrane MWCO the PEG rejection values must be plotted against the molecular weights of the PEGs studied. The PEG molecular weights reported in this work are the average molecular weights. The manufacturer's reported MW is often an average value as certain PEG MW normally contains a range of oligomers with the average value being reported. The

plots of rejection versus molecular weight are shown in Figures 3.20 and 3.21 below.

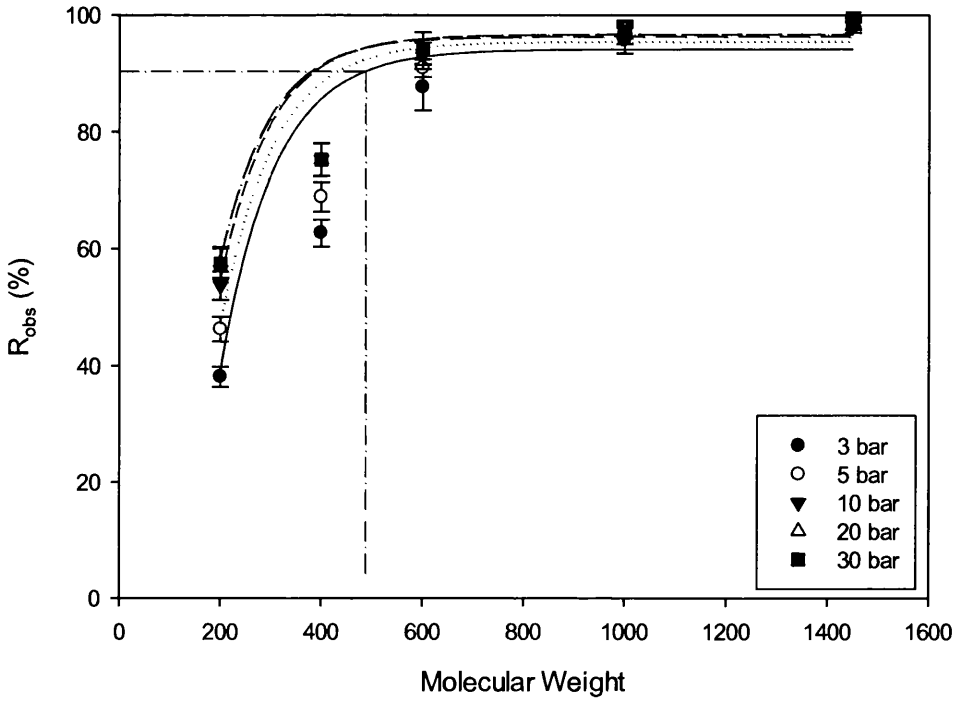

Figure 3.20: Observed rejection versus PEG molecular weight

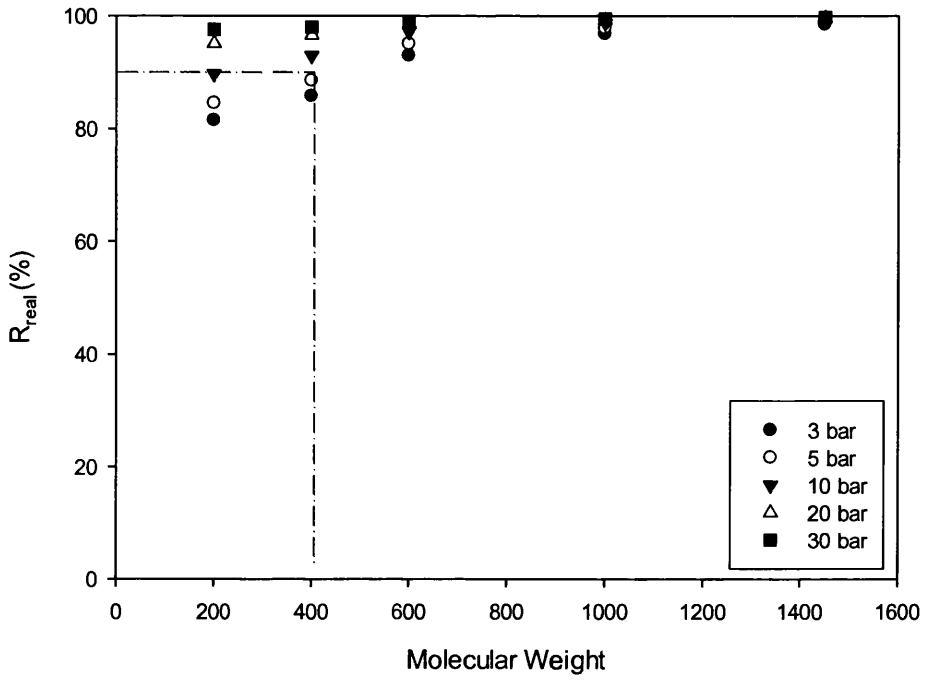

Figure 3.21: Real rejection versus PEG molecular weight

The data when plotted as rejection versus solute MW allows the MWCO of the membrane to be determined, although the rejection value for which MWCO is reported varies, however in this case a rejection value of 90% will be used. The MWCO values obtained from the observed and real rejection curves is 480 and 400 respectively. As both values are dependent on the same data with one corrected data set corrected for equipment mass transfer effects the NTR-7450 MWCO obtained in this study is 400 Da. This value lies at the lower end of the reported values as seen in Table 3.3.

Salt Rejection Experiments

Figure 3.22 displays the effect of varying concentration and pH on the rejection of NaCl at 10 bar. The NaCl rejection at 10^{-3}, 10^{-2}, 0.025, 10^{-1} M at 10 bar have been studied in this thesis. For all of the concentrations studied the rejection decreased with increasing concentration. The highest rejection was observed at 10^{-3} M where a 90% rejection was obtained at pH 6. This value is higher than any of the other values found in the literature.

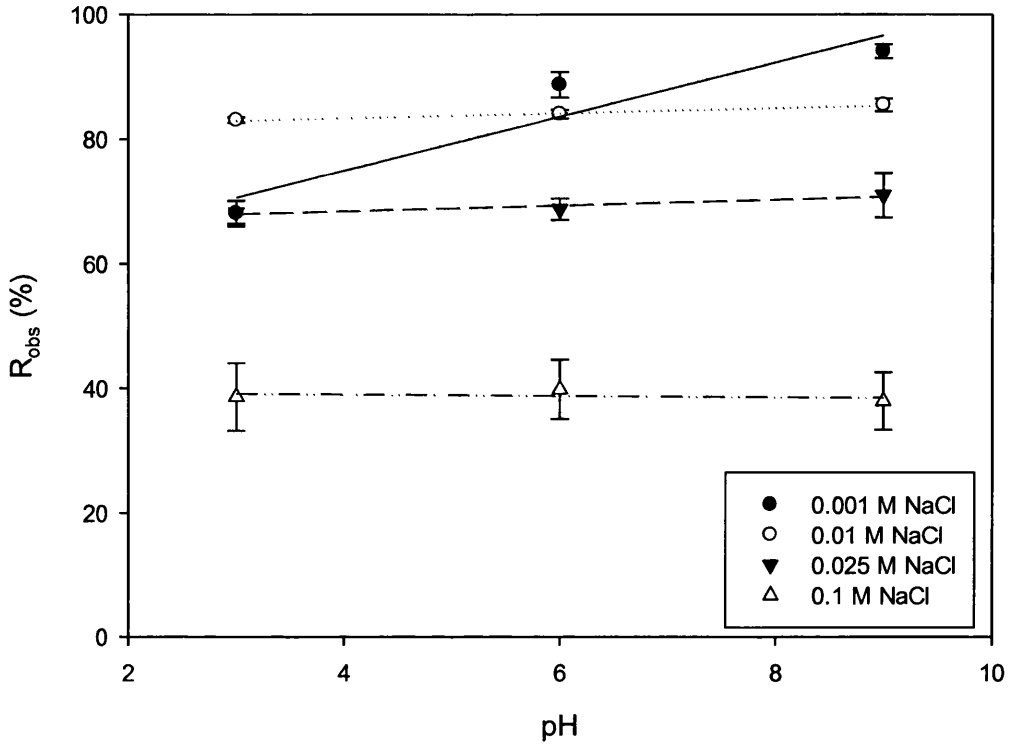

Figure 3.22: NaCl rejection versus pH at varying salt concentrations

A comparison can be drawn between the 0.01M rejection of NaCl where a observed rejection of 83% was achieved, and that of Nystrom et al. (1995) where a rejection value of 51% was obtained for a value of 0.008 M. The NaCl rejection decreases with increasing concentration due to the shielding effect of the cations on the charged membrane surface group (Afonso et al., 2001). The lowest observed NaCl rejection of 40% was seen at a concentration of 0.1 M. Interestingly, the effect of pH on the rejection is negligible for all the concentrations studied except at the lowest salt concentration (10^{-3}M) where at pH 3 there is a noticeable drop in rejection. This drop in rejection is due to the same phenomena as occurred at the lowest concentration in the zeta potential measurements where the concentration of the H^+ present exceeds 10% of the original ionic strength of the solution and therefore interferes with the NaCl rejection. The rejection of $CaCl_2$ at varying pH values is displayed in Figure 3.23. The rejection of $CaCl_2$ is lower than that of NaCl at similar molar concentrations with again the rejection decreasing with increasing concentration. The lower rejection of $CaCl_2$ when compared to NaCl is attributed to

the thickness of the electric double layer (Debye length) decreasing with the increasing ionic strength of the feed solution (Kukizaki, 2009). The rejection sequence of NaCl > $CaCl_2$ has been previously reported for the NTR-7450 membrane and is an example of the donnan exclusion mechanism (Schaep and Vandecasteele, 2001; Xu et al., 2011). The principle of this mechanism is that ions with the same charge sign as that of the membrane (co-ions) are excluded and cannot pass through the membrane in order to satisfy the condition of electro-neutrality, therefore an equivalent number of counter-ions are also retained, resulting in salt retention. However, in principle the ions with the opposite charge to that of the membrane (counter-ions) are able to permeate the membrane. In the case studied, Cl^- is the counter ion over the entire pH range studied and therefore exhibits high retention whereas the Na^+ counter ion permeates in order to maintain electro-neutrality. Interestingly, the NTR-7450 membrane exhibits an affinity towards Ca^{2+} ions and as the ion is the membranes counter-ion results in a reduction of the membranes negative charge. The overall consequence of this phenomenon is a decline in the rejection of the divalent salt as can be seen in Figure 3.22 and 3.23 where rejection decreased from above 80% to below 30% for 0.01M solutions of NaCl and $CaCl_2$ respectively.

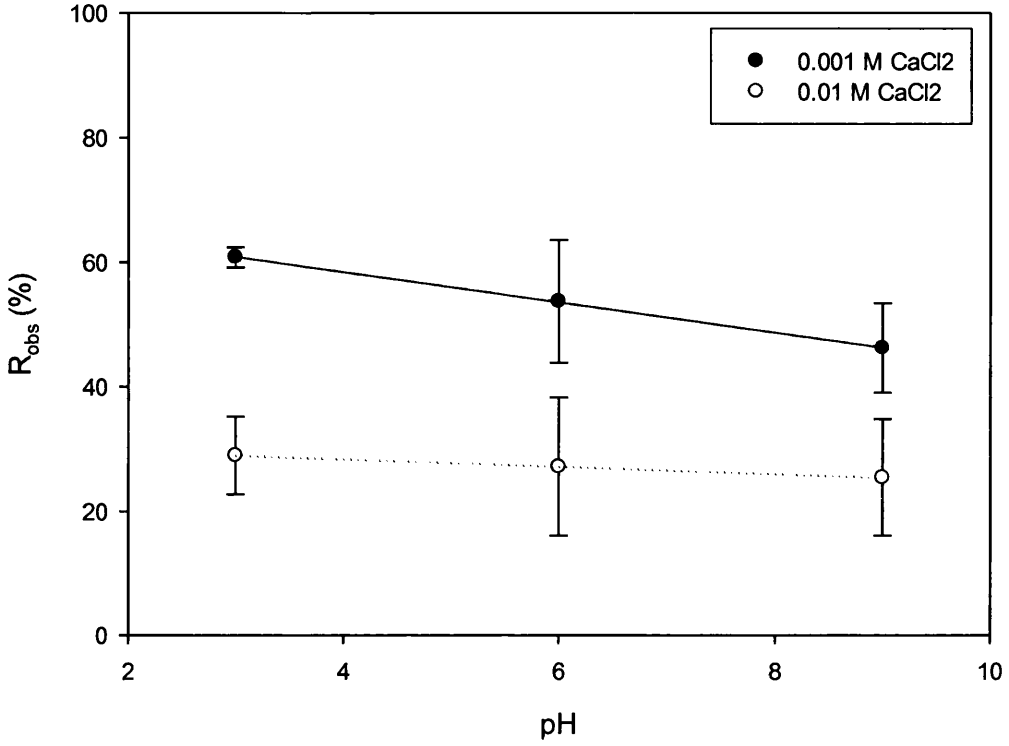

Figure 3.23: $CaCl_2$ rejection versus pH at varying salt concentrations

3.5.2.4 Conclusion

The NTR-7450 membrane has been characterised. The initial compaction study showed that the membrane required up to 2 hours of pre-compaction prior to starting any experimental work, however the membrane showed no signs of polymer relaxation once the pre-compaction had been undertaken. A membrane flux value of 3.2 LMH/bar was obtained during the experimental work a value which is located at the lower end of those reported in the literature. The MWCO obtained through this experimental work for the NTR-7450 membrane is 400 Da. A salt rejection of 83% was obtained at a concentration of 0.01M, higher than the value often reported in the literature of 50% at similar conditions. The membrane was characterised in frontal filtration mode and the effects of mass transfer were accounted for. Therefore, should the membrane be characterised in cross flow mode, as lo9ng as the effects of mass transfer are understood the characterisation experiments should produce the same results.

Overall this characterisation has in essence added more fuel to the fire surrounding the properties of the NTR-7450 membrane. This work has highlighted the need for an accurate and universal characterisation method. The result of each research group and manufacturer using unique characterisation methods results in a wide variety of data being available in the literature making membrane selection to the end user an almost impossible task without extensive laboratory trials. A simple yet comprehensive method for characterisation should at least allow for membrane properties to be determined similarly across each manufacturer and research group and hopefully results in an agreement over membrane characteristics. However, there is a slight discrepancy noticed in this work. The variation of pure water flux between each of the membranes studied suggests that using small membrane areas cut from a larger sheet results in variations between each membrane. This may be an inherent error in manipulating industrial membrane modules to be used at a laboratory scale. Therefore a comparison between properties of laboratory scale membrane sheets and industrial scale modules should be investigated as well as further characterisation of membranes at a laboratory scale.

Appendix A3: Supporting information for Mass transfer characterisation of membrane cells.

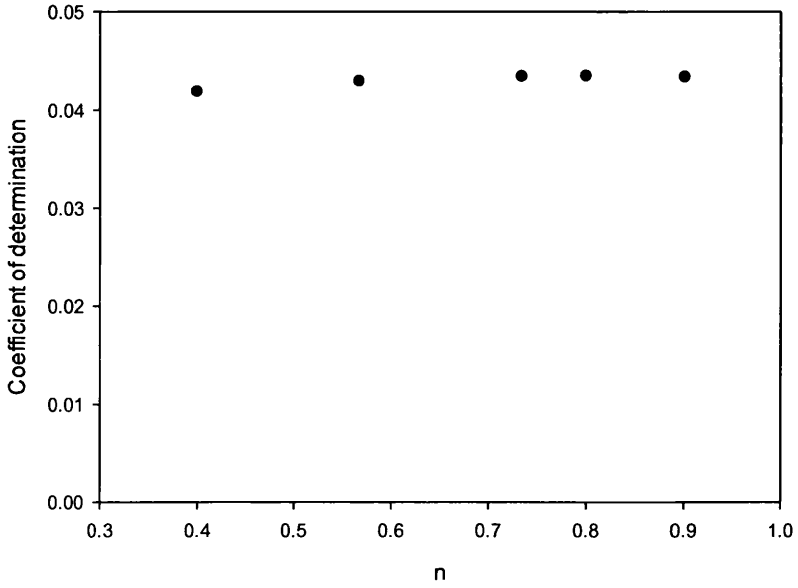

Figure A3.1: Optimisation of the coefficient of determination for the Amicon Cell

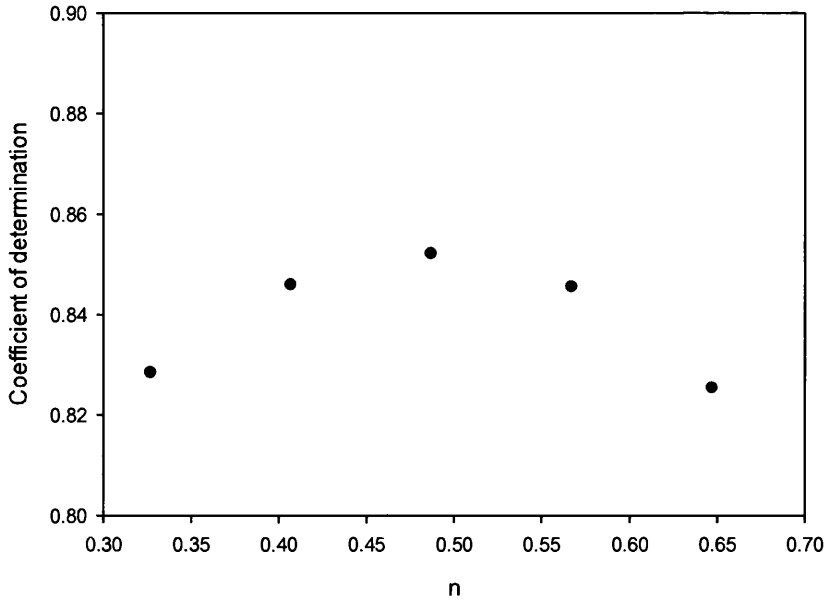

Figure A3.2: Optimisation of the coefficient of determination for the Sterlitech Cell

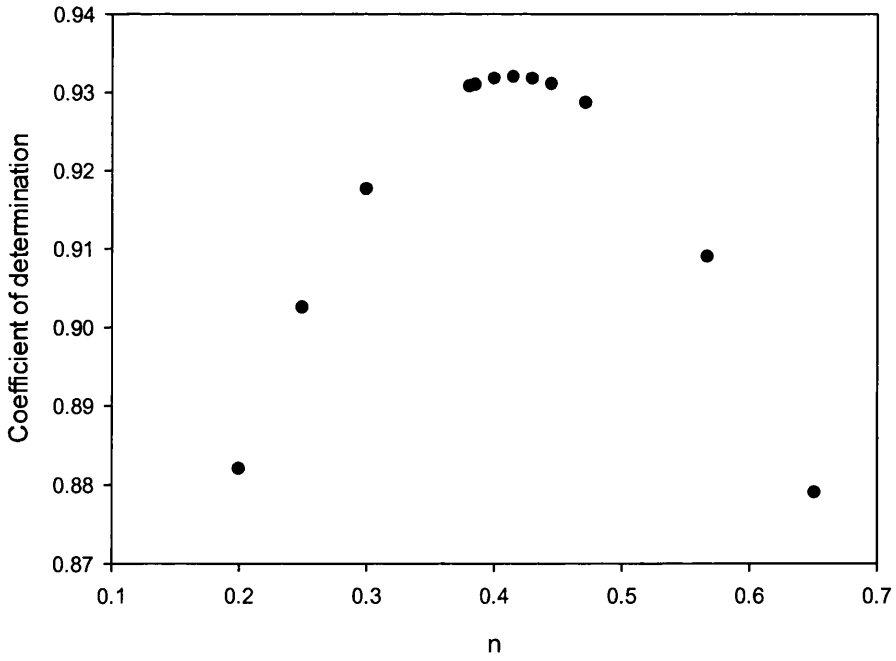

Figure A3.3: Optimisation of the coefficient of determination for the Membranology Cell

Figures A1.1, A1.2 and A1.2 were used for the determination of the empirical constant, n of the three cells studied for the mass transfer study in Section 3.5.1

Appendix A4: Experimentally derived hindrance factors with varying pore size (pore radius variation of ± 0.2 nm).

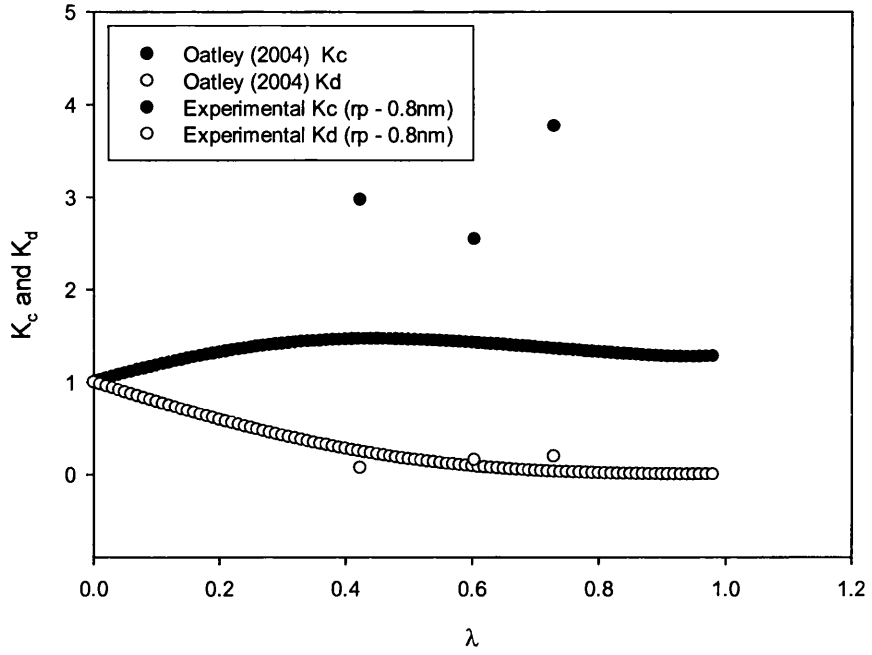

Figure A4.1: Experimentally derived hindrance factors with a pore radius of 0.8nm

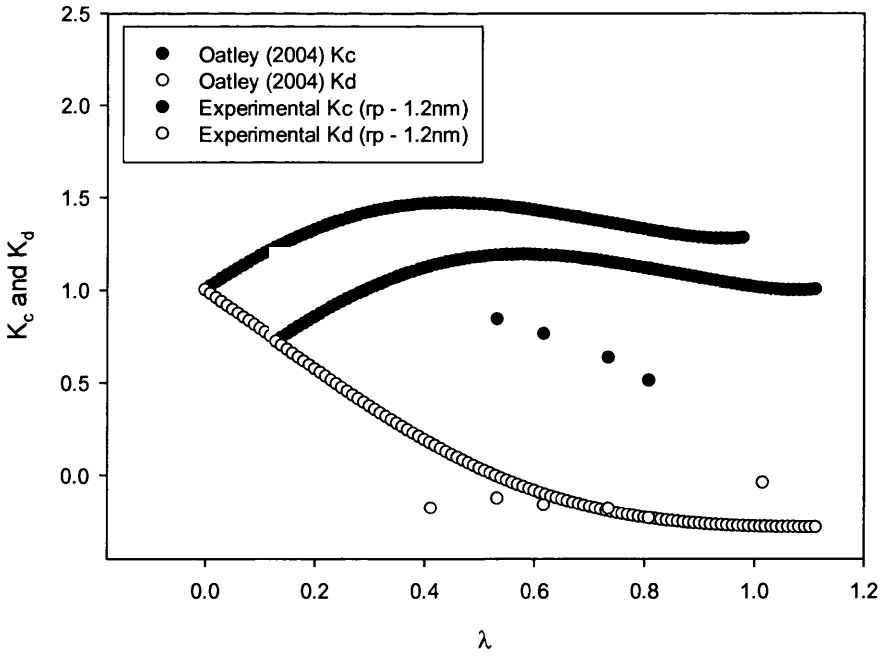

Figure A4.2: Experimentally derived hindrance factors with a pore radius of 1.2nm

Figures A4.1 and A4.2 show the effect of varying the pore radius by ± 0.2nm for the hydrodynamic drag study shown in Section 4.1. The figures show significant deviation away from the theoretical correlation for both the Kc and Kd values. The relationship displayed in Figure 4.2.8 displays good agreement between that of the theoretical and experimentally obtained values suggesting that the experimentally determined membrane pore size is correct.

Appendix A5: GC chromatograms for calystegine detection and quantification

The retention times reported vary due to modification of the GC equipment (column maintenance. All GC chromatogram peaks were checked against known mass spectra for confirmation. Due to the number of samples run only a snapshot of the raw data is presented.

Storage Study

Retention Times

Castanospermine - 11.74 min

Calystegine B2 - 11.06 min Chromatograms shown are of whole potatoes stored in cold light conditions (CLW).

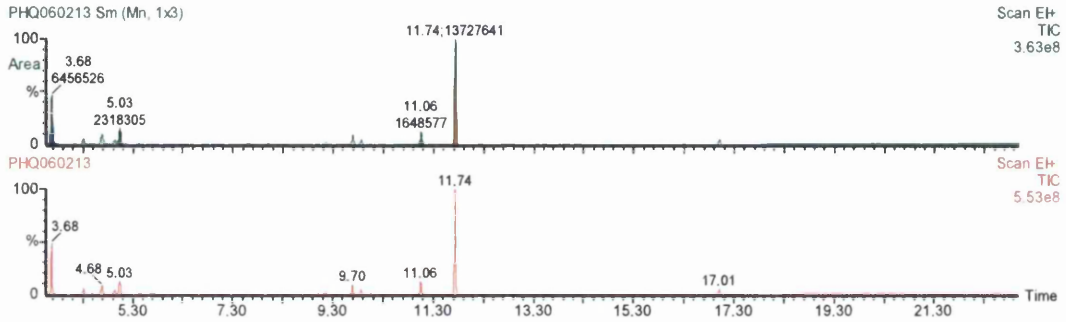

Figure A5.4: Chromatogram of sample 1CLW

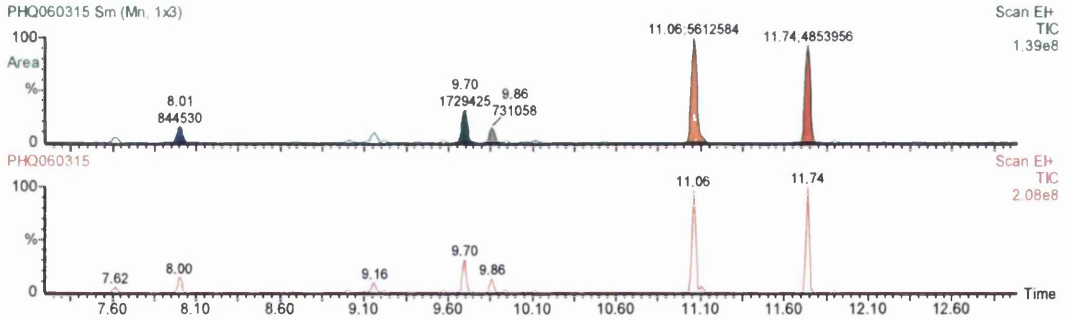

Figure A5.5: Chromatogram of sample 2CLW

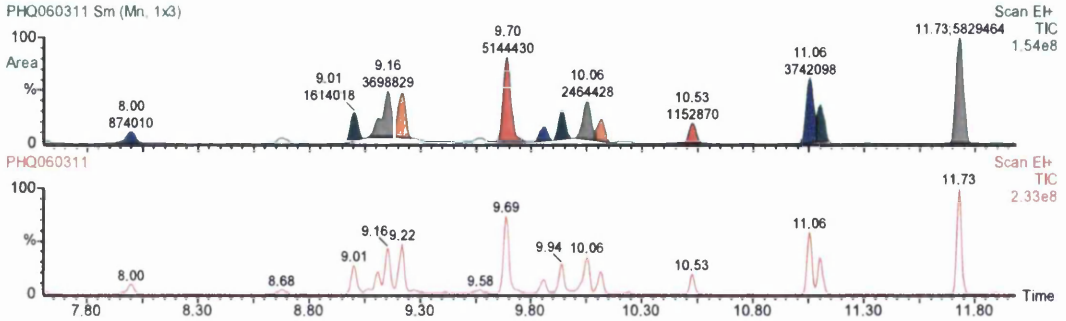

Figure A5.6: Chromatogram of sample 3CLW

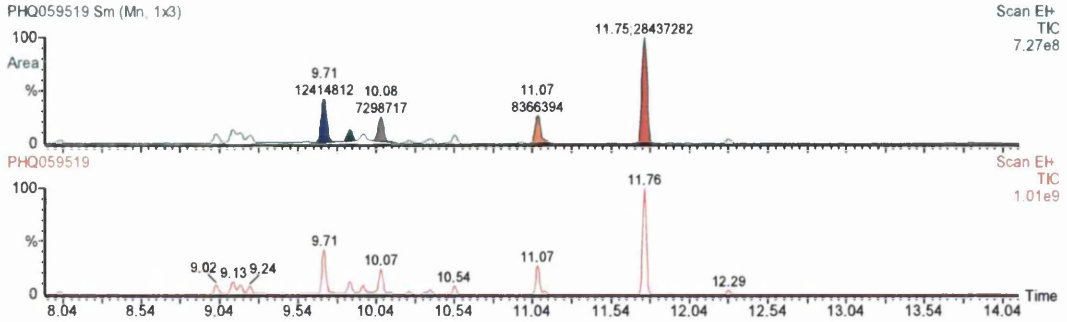

Figure A5.7: Chromatogram of sample 4CLW

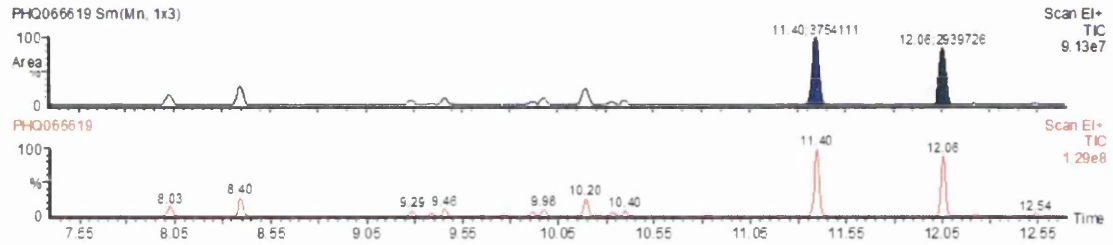

Figure A5.8: Chromatogram of sample 8CLW

Pilot Scale Processing

Retention Times

Castanospermine - 12.08 min

Calystegine B2 - 11.40 min

Calystegine B4 - 9.7 min

Calystegine A3 - 8.02 min

Calystegine A5 - 8.39 min

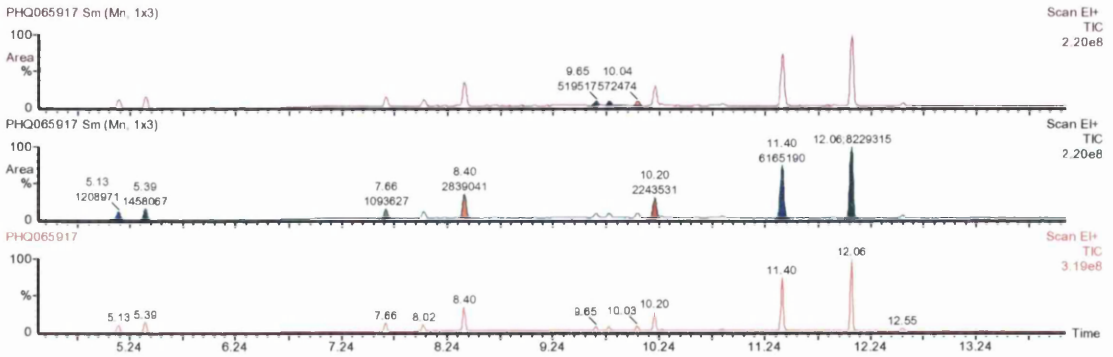

Figure A5.9: Chromatogram of RO concentrate

Mass Spectra

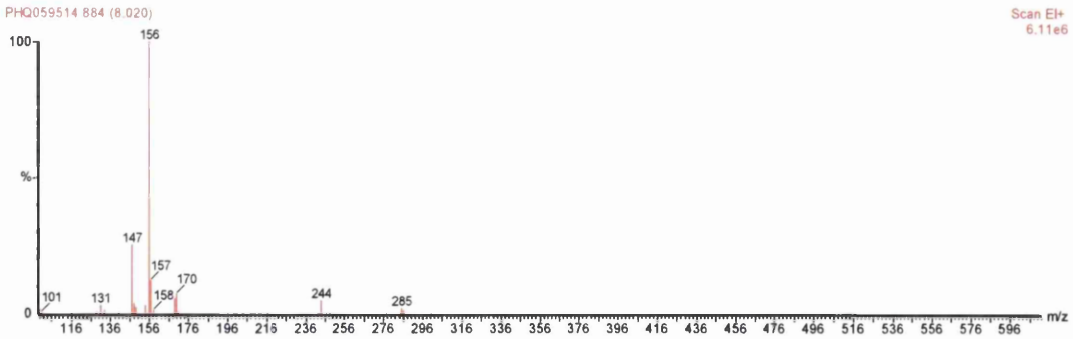

Figure A5.1: Mass Spectra of Calystegine A3

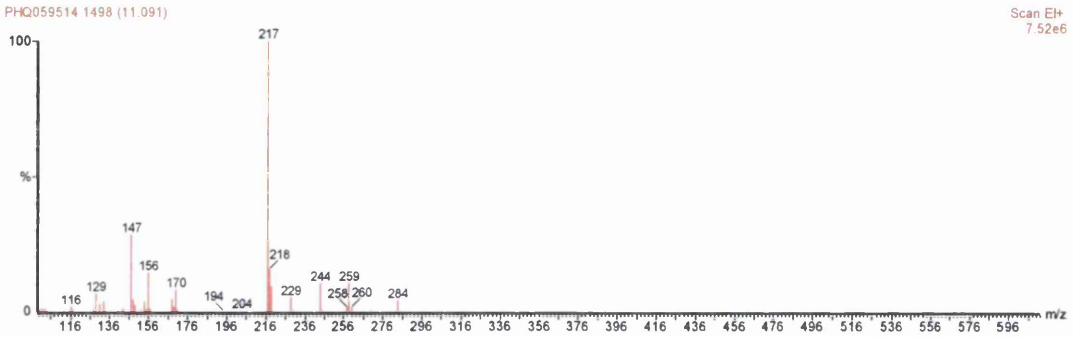

Figure A5.2: Mass Spectra of Calystegine B2

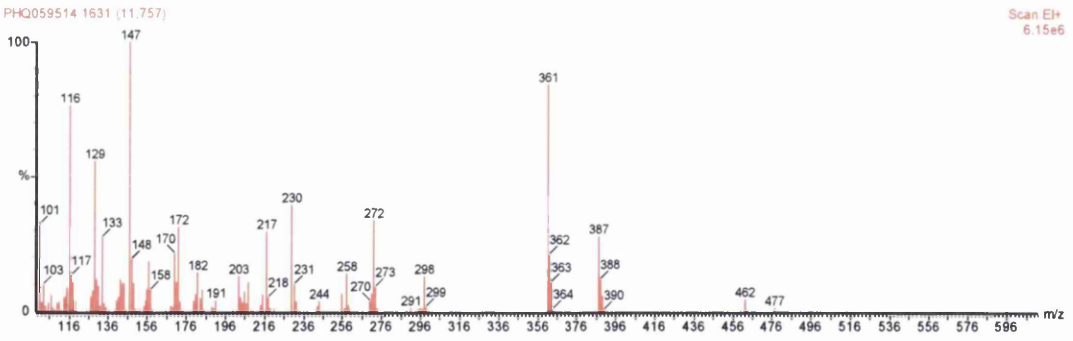

Figure A5.3: Mass Spectra of Castanospermine

Appendix A6: Timeline of PhD Research Undertaken

External project with Sponsor Company (KESS Requirement) started 18 months into PhD.

Table A6.1: Timeline of PhD Research Undertaken

Start Date July 2011	Year 1 (Quarter)				Year 2 (Quarter)				Year 3 (Quarter)				Year 4 (Quarter)	
Activity	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**
Literature Review														
Learning necessary biological techniques														
Fungal Bioactives														
Equipment Mass Transfer Study														
Membrane Drag Study														
Di-electrics Research														
Characterisation of NTR-7450 membrane														
Potato Bioactive Research (PhytoQuest Ltd)														
Thesis Write up														
Viva														

Appendix A7: Critical appraisal of current nanofiltration modelling strategies for seawater desalination and further insights on dielectric exclusion.

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Critical appraisal of current nanofiltration modelling strategies for seawater desalination and further insights on dielectric exclusion

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HIGHLIGHTS

- A critical appraisal of seawater desalination modelling using the extended Nernst–Planck equations is made.
- A lack of fundamental understanding and physical properties information is highlighted.
- Experimental investigation at the membrane isoelectric point indicates dielectric screening may be taking place.

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ABSTRACT

Adequate fresh water supply is an increasing global issue as water stress continues to grow in many areas of the planet. Seawater desalination processes are essential to supply domestic needs as well as those of industry and agriculture. Membrane processes are now the dominant technology for desalination and will continue to grow in use for the foreseeable future. Accurate methods for the design, development and scale-up of salt water desalination plants are a vital tool for developing cost effective and sustainable plants. In this paper, the current best practice for modelling nanofiltration and reverse osmosis using the extended Nernst–Planck equation is discussed and a critical appraisal is made. The role of dielectric exclusion from nanofiltration membranes is discussed and experimentation at the membrane isoelectric point indicates that screening of this phenomenon may be occurring. The paper highlights the need for a rigorous evaluation of membrane modelling for seawater desalination, especially for multi-component and concentrated salt solutions.

1. Introduction

Water is a vital commodity with approximately 3 billion people around the world having no access to clean drinking water [1]. According to the World Water Council the planet will be around 17% short of the fresh water supply needed to sustain the world population by 2020 [2]. The majority of the earth's water is contained in the oceans (~97%), while another 2% is trapped in icecaps and glaciers, resulting in less than 1% being accessible as fresh water [3,4]. The oceans represent a virtually unlimited supply of water, although seawater itself is unsuitable for human consumption and industrial/agricultural uses without treatment. For this reason, desalination has become an important method for the production of fresh water with the daily desalination capacity estimated as 71.9 million m^3/day at the end of 2011 [5] and demand continues to grow as water stress increases. Patents filed in 2010 for desalination technologies are double than that of 2005, demonstrating the increasing interest and research activity in this field [6]. The desalination process is considered to be detrimental in terms of environmental impact and cost, these factors have been reviewed previously [7,8]. Therefore, process selection, understanding and optimisation are vital for successful desalination operations and modelling plays an important role in each of these aspects.

Desalination technologies and methods have also been reviewed [3,9] and are predominantly thermal processes, membrane processes and hybrid systems. High pressure membrane technologies such as nanofiltration (NF) and/or reverse osmosis (RO) membranes are the most effective desalination methods [10] and RO (often referred to as seawater reverse osmosis – SWRO) has become the most internationally widespread desalination technology [11]. In the period 2005–2008, the annual worldwide contracted capacity of RO increased from 2.0 million to 3.5 million m^3/day [12] which represents over 50% of the total installed desalination capacity on the planet [13,14].

NF is a pressure driven membrane separation process with characteristics between those of reverse osmosis and ultrafiltration and plays an important role in the desalination process. NF membranes are typically polymeric, asymmetric, and consist of a low resistance support layer with a functionally active porous top layer [15,16]. The

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nominal molecular weight cut-off of an NF membrane is in the range 100–1000 Da, indicating that the NF membrane's active layer has an approximate pore size of 1 nm. NF presents many advantages over reverse osmosis, such as lower operating pressures, higher fluxes, and lower investment, operation and maintenance costs. NF exhibits high rejection for multivalent salts, resulting in a suitable pre-treatment for seawater desalination [17–19]. Additionally, NF may help improve the RO membrane flux [20]. The pre-treatment of seawater using NF was first proposed by Hassan et al. [21] where NF was utilised as a pre-treatment for both membrane and thermal desalination techniques resulting in lower potable water production costs, compared to no pre-treatment. In 2002, an estimated 65% of the NF market was in water treatment [22,23].

NF is an extremely complex process and is dependent on the micro-hydrodynamic and interfacial events occurring at the membrane surface and within the membrane nanopores. The nano-scale phenomena involved in uncharged solute and salt separations by NF are extremely complex and, as such, likely to be a rigorous test of any macroscopic description of ion transport and partitioning. The transport of uncharged solutes is reasonably well established through numerous studies of UF membranes. There have been many works over the past thirty years on modelling the transport of charged solutes across a charged membrane. A large number of predictive NF models have been based on either the charged capillary model [24], models based on the extended Nernst–Planck equation [25] or the irreversible thermodynamic model [26]. The application of the extended Nernst–Planck equation was originally proposed by Schlogl (1966) [27] for the description of transport of electrolytes in RO through ion-exchange membranes and is arguably the most commonly used model of modern times. The equation is particularly useful for NF as consideration is given to the mechanisms of transport and the adjustable fitting parameters required are based upon real measurable membrane properties. Rejection from NF membranes may be attributed to a combination of both steric and non-steric effects. The fact that the dimensions of the NF active layer are near atomic length scales, coupled with limitations in current measurement technologies, has delayed a detailed knowledge of the physical structure and electrical properties of real NF membranes and has resulted in uncertainty and significant debate over the true nature of the separation mechanisms [15] and the role of dielectric exclusion is particularly contested [28].

The aim of this work is to outline the state-of-the-art in NF modelling and critically assess the current methodology when considering seawater separations. In addition, the work will also introduce some new insights on the phenomena of dielectric exclusion and will show that our current understanding needs attention by considering the rejection of concentrated NaCl solutions from a membrane at the pH corresponding to the membrane isoelectric point.

2. Relevant theory

The prediction of membrane performance has been an active area of research over the last two decades. During that time, the emphasis has shifted from empirical black box models based on irreversible thermodynamics [26] to models based on the extended Nernst–Planck equation [25] due to the ability of the latter to provide information related to properties of both the membrane and the process stream. The main purpose of such models is to incorporate as much physical realism of the membrane process as possible in order to better match measurable quantities to adjustable model parameters. One should always be mindful that the major limitation of nanofiltration modelling is the requirement for characteristic model parameters, such as pore radius and membrane charge, that are not readily measured at the near atomic length scales encountered. Similarly, the development of rigorous physical descriptions (such as Molecular Dynamics simulations) has been limited by the lack of detailed knowledge of the physical structure and electrical properties of real nanofiltration membranes and process streams. As a direct consequence, developments in modelling have moved in parallel with improvements in the measurement techniques employed for the characterisation of nanofiltration membranes and process streams, as only then will a check of the appropriateness of model parameters be possible.

The transport of ions is typically described using the extended Nernst–Planck equation

$$j_i = -\frac{c_i K_{i,d} D_{i,\infty}}{RT}\frac{d\mu}{dx} + K_{i,c} c_i V \tag{1}$$

where j_i is the ionic flux, c is the concentration, V is the solvent velocity and $K_{i,c}$ and $K_{i,d}$ are hindrance factors to account for the convection and diffusion inside a confined space, respectively. The electrochemical potential is written as

$$\mu_i = RT \ln a_i + V_{si} P + z_i F \psi + \text{constant} \tag{2}$$

where R is the universal gas constant, T is the absolute temperature, V_{si} is the specific volume of the ion, P is the operating pressure, z is the ion valence, F is the Faraday constant and ψ is the electrical potential. Differentiation of Eq. (2) results in

$$\frac{d\mu}{dx} = \frac{RT}{c_i}\frac{dc_i}{dx} + \frac{RT}{\gamma_i}\frac{d\gamma_i}{dx} + V_{si}\frac{dP}{dx} + z_i F \frac{d\psi}{dx}. \tag{3}$$

Substitution of Eq. (3) into Eq. (1) yields

$$j_i = -D_{i,p}\frac{dc_i}{dx} - \frac{c_i D_{i,p}}{\gamma_i}\frac{d\gamma_i}{dx} - \frac{c_i D_{i,p}}{RT} V_{si}\frac{dP}{dx} - \frac{c_i D_{i,p}}{RT} z_i F \frac{d\psi}{dx} + K_{i,c} c_i V. \tag{4}$$

Eq. (4) represents the full extended Nernst–Planck equation and governs the transport of an ion in solution. In this case the equation has been specifically developed to describe the transport of an ion through a membrane pore, typical of that found in nanofiltration and loose reverse osmosis membranes. In order to solve the transport equation, the solute concentration at the feed side and the permeate side, $c_i(0)$ and $c_i(\Delta x)$, of the membrane must be known. The entrance and exit of an ion from such a pore is an equilibrium process and this relationship is expressed as

$$\frac{\gamma_i c_i}{\gamma_i^o C_i} = \Phi_i \exp\left(-\frac{z_i F}{RT}\Delta\psi_D\right)\exp\left(-\frac{\Delta W_i}{k_B T}\right). \tag{5}$$

The first two terms on the right hand side of Eq. (5) are the classic expressions for both steric and Donnan effects respectively [29–31] and are generally well accepted throughout the literature [28]. The third term on the right hand side of Eq. (5) represents partitioning as a result of dielectric exclusion. There has been much debate over the nature of this effect and this has been summarised in a recent review [28] and will be further discussed later in this paper.

The typical method for solving the equilibrium equation [Eq. (5)] is to assume ideal conditions and that electroneutrality exists in the bulk solution and inside the membrane pore, i.e.

$$\sum_{i=1}^{n} z_i C_i = 0 \text{ and } \sum_{i=1}^{n} z_i c_i = -X_d \tag{6}$$

where X_d is the effective membrane charge density. When the assumptions are met, one may equate the Donnan potential for each ionic species and rearrange Eq. (5) in terms of a particular ionic species, in this case species 1

$$c_{i(0)} = C_{i,w}\Phi'_i\left(\frac{c_{1(0)}}{C_{1,w}\Phi'_1}\right)^{\frac{z_i}{z_1}} \tag{7}$$

and then substitute the result into Eq. (6)

$$z_1 c_{1(0)} + z_2 C_{2,w} \Phi'^{\frac{z_2}{z_1}}_2 \left(\frac{c_{1(0)}}{C_{1,w}\Phi'_1}\right)^{\frac{z_2}{z_1}} + \ldots + z_n C_{n,w} \Phi'^{\frac{z_n}{z_1}}_n \left(\frac{c_{1(0)}}{C_{1,w}\Phi'_1}\right)^{\frac{z_n}{z_1}} + X_d = 0 \tag{8}$$

where $\Phi'_i = \Phi_i \exp\left(-\frac{\Delta W_i}{k_B T}\right)$.

Thus, for a known feed solution, the pore entrance concentrations can be calculated from the solution of Eq. (8) and substitution to Eq. (7) for all ions. The same situation exists at the exit of the pore. The typical method for solution of the transport equation is to assume ideal conditions and neglect the effect of pressure on the chemical potential. When this is the case Eq. (4) can be simplified and manipulated to give

$$\frac{dc_i}{dx} = \frac{V}{D_{i,p}}\left[K_{i,c}c_i - C_{i,p}\right] - z_i c_i \left[\frac{\sum_{i=1}^{n} \frac{z_i V}{D_{i,p}}\left[K_{i,c}c_i - C_{i,p}\right]}{\sum_{i=1}^{n} z_i^2 c_i}\right]. \tag{9}$$

The solution procedure is then to numerically solve Eq. (9) for each ionic species and iterate the solution based on estimations of the permeate concentrations using Eq. (8) at the pore exit as a check mechanism. In the case of a neutral species, Eq. (9) simplifies to form an algebraic result

$$R = 1 - \frac{C_{i,p}}{C_{i,w}} = 1 - \frac{K_{i,c}\Phi_i}{1 - \left[1 - K_{i,c}\Phi_i\right]\exp(-Pe)} \tag{10}$$

where $Pe = \frac{K_{i,c}}{K_{i,d}} \frac{r_p^2}{8\eta D_{i,\infty}} \Delta P_e$ is a modified Peclet number, ΔP_e is the effective pressure driving force ($\Delta P - \Delta\Pi$), η is the pore viscosity and r_p is the pore radius.

3. Materials & methods

3.1. Experimental set up for membrane rejection experiments

All rejection experiments were carried out at laboratory scale using a frontal filtration set-up illustrated in Fig. 1. The system consisted of a customised membrane cell supplied by Membranology (Membranology Ltd., UK). The details of this cell are not available for publishing at the present time, however, the cell may be thought of as a standard Amicon type cell capable of high pressure operation.

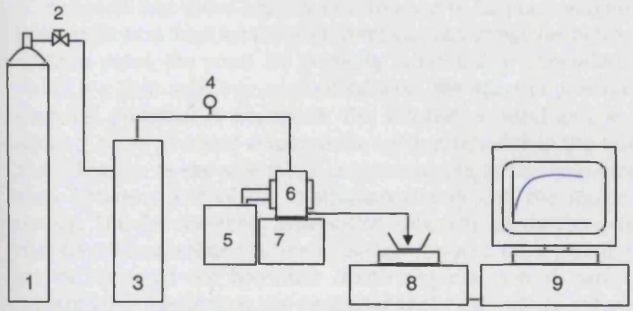

Fig. 1. Outline of experimental set-up. (1) nitrogen cylinder, (2) valve, (3) 1.5 L reservoir, (4) pressure sensor, (5) water bath, (6) Membranology cell, (7) magnetic stirrer, (8) electronic balance, (9) PC.

The active membrane area in the cell is 0.00418 m^2. Feed solution is placed within the cell and the system is then pressurised with oxygen free nitrogen (BOC, UK). The applied pressure is adjusted via a regulator connected to the nitrogen cylinder and is monitored using a pressure sensor (Druck DPI 104 – RS Components, UK). All membrane experiments were carried out at 25 ± 0.1 °C using a stirrer speed of 300 rpm. Permeate flow rate was measured via the mass output of the cell monitored by a balance (Ohaus Navigator N24120 – Ohaus Europe, Switzerland) and recorded by a PC. Samples of the permeate stream were collected for analysis after 15 mL of permeate had been removed.

3.2. Membrane and feed solutions

The membrane used in this study was the Desal DK (GE Osmonics, USA). This membrane is a thin film composite with a polyamide active layer. The membrane was cut to size and immersed in deionised water for at least 24 h prior to use. Prior to any membrane rejection experiments, the membrane was flushed with deionised water for 1 h at 30 bar in order to negate any compression effects. NaCl was obtained in high purity form (Fisher Scientific, Loughborough, UK) and salt solutions were made up with deionised water to the required concentration. The salt concentrations used for the experimental work were 0.000017, 0.0001, 0.00017, 0.0017, 0.00025, 0.001, 0.017, 0.01, 0.1, and 0.6 M and were selected to cover a wide range typical of academic studies and relevant for industrial purposes. NaCl has been selected as this represents the bulk species within seawater and this range of concentration is the equivalent of 1 to 35,000 ppm with the highest concentration typical of seawater. Rejection was calculated from conductivity measurements of the feed and samples of the permeate. All conductivity measurements were performed at 25 °C using a Russell RL060C conductivity meter and probe (Fisher Scientific, Loughborough, UK). pH was measured using an IQ 150 pH meter and probe (Spectrum Technologies Inc., Illinois, USA).

3.3. Isoelectric point determination

The zeta potential (ζ-potential) was determined using an electrokinetic analyser (EKA, Anton Paar, Graz, Austria) based on the streaming potential method [32]. Flat sheets of the Desal DK membrane were studied over a range of pH and concentrations in order to generate a zeta profile for the membrane from which the isoelectric point is determined. The ζ-potential was calculated using the Smoluchowski equation. Previous studies have shown that the Desal DK membrane has an isoelectric point in the region of pH 3.5 to 4.1 [32–35].

The membrane isoelectric point was additionally determined from salt rejection studies at varying pH values. The feed solution pH was adjusted by the addition of 0.1 M HCl or 0.1 M NaOH, both obtained from Fisher Scientific (Fisher Scientific, Loughborough, UK). The pH was varied between pH 3 to 10 using each of the concentrations stated previously. The isoelectric point was determined from the minimum rejection value obtained over the range of pH at fixed concentration and a constant applied pressure of 10 bar.

3.4. Calculation of rejection

The experimentally observed rejection is defined as

$$R_{obs} = 1 - \frac{C_p}{C_f} \tag{11}$$

where C_p and C_f are the concentrations of the permeate and feed respectively. In reality, due to concentration polarisation this definition of rejection is not accurate because the concentration at the membrane surface, C_w is higher than the bulk feed concentration; C_f. The real

rejection of the solute, R_{real}, which is always equal to or higher than R_{obs} is defined as

$$R_{real} = 1 - \frac{C_p}{C_w} \tag{12}$$

The concentration at the wall, C_w, cannot be measured directly and therefore must be calculated indirectly with a suitable model for concentration polarisation. The approach to concentration polarisation taken in this study is that of the infinite rejection method described previously [36,37]:

$$\exp\left(\frac{J_v}{k}\right) = \frac{C_w - C_p}{C_f - C_p} \tag{13}$$

where J_v is the volumetric flux through the membrane and k is the mass transfer coefficient in the polarised boundary layer. This result is equally applicable to a system of uncharged solutes. The exponential term represents the mass transfer effect and shows that when $C_w = C_f$, the exponential term becomes unity, and when concentration polarisation is occurring the exponential term becomes greater than unity. The mass transfer coefficient may be determined experimentally by linearization of Eq. (13), yielding

$$\ln\left(\frac{1-R_{obs}}{R_{obs}}\right) = \frac{J_v}{k} + \ln\left(\frac{1-R_{real}}{R_{real}}\right). \tag{14}$$

In this case, the mass transfer coefficient is expressed as

$$k = a\omega^n \tag{15}$$

where a and n are constants and ω is the membrane cell stirrer speed. Therefore, the real rejection may be determined from the experimentally observed rejection on extrapolation to infinite stirrer speed by plotting $\ln[(1 - R_{obs}) / R_{obs}]$ against J_v / ω^n. The slope of the best fit line will be equal to $1/a$. The real rejection may then be calculated by rearranging Eq. (14) to yield

$$R_{real} = \frac{1}{\exp\left(\ln\left[\frac{1-R_{obs}}{R_{obs}}\right] - \frac{J_v}{a\omega^n}\right) + 1}. \tag{16}$$

4. Results and discussion

4.1. Consideration of theoretical aspects

When using any model, the scientist or engineer should always be aware of the inherent assumptions made during the development of the model and those assumptions imposed to facilitate solution. In this specific case there are many assumptions, including: the membrane contains pores, the pores are perfectly cylindrical in dimension, the solute is a rigid sphere in an ideal solution, the effect of pressure on chemical potential is negligible, the solution is ideal and so on. Many of these standard assumptions are questionable in the case of nanofiltration as the size range considered sits at the near atomic scale between that of the continuum theory and the molecular theory. The fundamental assumption inherent to the models of nanofiltration is related to the presence of pores. Oatley et al. [28] briefly reviewed the literature concerning the porous nature of nanofiltration membranes and concluded that, based on the volume of evidence available indicating that pores may be present, this is a reasonable assumption. However, the fact that nanofiltration membranes have distinct cylindrical pores is highly questionable. AFM studies have provided images that are considered to be nanofiltration pores [19,38,39] and, as a whole, these are predominantly slit like in nature rather than circular at the pore entrance. Similarly, detailed SEM images of polymer active layers in ultrafiltration membranes show that the pathway along the pore is quite convoluted and not the ideal cylinder [40,41]. However, the relevance and effect of pore tortuosity on rejection and flux from nanofiltration membranes have never been systematically considered when modelling with the Nernst–Planck equation. The assumption that ideal conditions exist inside the nanopore is often made as considering the changes in activity coefficient not only increase the complexity of Eq. (4) hindering solution, but also introduce further adjustable parameters that are difficult to determine experimentally. In addition to this, fundamental understanding of the physical properties and phenomena occurring in a confined space is limited and even simple questions such as what is the extent of ion hydration inside a nanopore are not trivial [42]. Similarly, surface interactions of the membrane with the solvent phase are not well understood and the impact of water structuring on physical variables such as the dielectric constant of the pore, the diffusion coefficient and viscosity is not exactly known. There are several papers available in the literature that suggest that electroneutrality [Eq. (6)] is not valid within the nanopore [43,44]. Therefore, there is still a great deal of questions to answer when considering nanofiltration modelling.

Possibly the greatest issue when considering the current application is the seawater itself. Seawater is a very complex media and has an immeasurable number of constituents and varies in concentration from ocean to ocean. For example, seawater concentration is typically accepted to be around 35,000 ppm. However, in estuarine waters this may be as low as 20,000 ppm and in the Red Sea, seawater concentration has been measured at 42,000 ppm. The generic salt make up of seawater is generally considered to be a cocktail of major constituents, trace constituents, nutrients, dissolved gasses and organics. The major constituents are salts comprised of chlorine (Cl^-), sodium (Na^+), sulphate (SO_4^{2-}), magnesium (Mg^{2+}), calcium (Ca^{2+}), potassium (K^+) and carbonate (HCO_3^-) in order of abundance and account for around 99% of the total dissolved salts. Some other minor salts worthy of note are bromine (Br^-) and strontium (Sr^{2+}), although almost all of the naturally occurring 92 elements may be found in sea water following rigorous analysis. Thus, exact modelling of such a complex fluid is inherently difficult and only a small number of studies exist that have even attempted to be part of this challenge [45,46]. The extended Nernst–Planck equation can in essence be used to model the transport of an infinite number of ions and dissolved species through the nanofiltration membrane. However, the overwhelming majority of modelling studies to date have considered only very dilute and idealised solutions typically containing a maximum of only 2, 3 or sometimes 4 ions. If accurate sea water modelling is to become common place then more effort needs to be placed into modelling systems of real industrial importance, i.e. multiple ions and solutes at high concentrations. In order for this to be possible, then new approaches need to be developed in order to understand the complex interactions and physical phenomena such as membrane charge in such systems. Similarly, fresh approaches need to be developed for solution of the intensive mathematics that will result from such systems. A recent model that illustrates the kind of approach needed is that of Yaroshchuk et al. [47]. Although focussed on the solution-diffusion model, this paper looks at the separation of ions as a dominant salt amongst a series of trace ions. In principle, one could draw parallels to the short cut method known as the key components methodology for designing fractional distillation columns which are extremely complex in nature. The authors concluded that the approach taken could be useful for the optimisation of nanofiltration for mixed electrolyte solutions in various applications. The challenge for the future of nanofiltration modelling must be to gain further understanding of the fundamental phenomena occurring during the nanofiltration process in

order to not only find answers to the questions posed above, but also develop accurate yet simplified models and design methodologies for seawater desalination for use by non-specialist scientists and engineers.

4.2. Confirmation of the membrane isoelectric point

Pore dielectric effects and effective membrane charge density normally exhibit a coupled behaviour. Thus, in order to evaluate a single effect their relationship must be decoupled. The membrane isoelectric point provides an opportunity to study only dielectric effects due to the membrane charge density being effectively neutralised. The results of the streaming potential measurements are shown in Fig. 2a. The profile of the ζ-potential for the salt studied exhibits positive values at low pH, becoming negative as pH rises and exhibits a negative plateau at high pH. The magnitude of the ζ-potential is proportional to concentration at high pH, with higher ζ-potential values observed at lower salt concentrations. The isoelectric point for each of the salt concentrations studied was in the range from pH 3.0 to 4.2, which is consistent with previous studies [32,33,35]. Interestingly, the isoelectric point appears to become lower as the concentration of salt increases. This suggests that a small quantity of the chloride ions in solution are adsorbing to the membrane surface. The ζ-potential versus chloride ion concentration in solution is illustrated in Fig. 2b at pH 4.0 (data obtained by interpolation from experimental results). This information indicates that the ζ-potential at this pH is proportional to chloride ion concentration and, when taking experimental error into consideration, is linear in nature. This trend illustrates that chloride ion adsorption is taking place and that the shift in the isoelectric point is real and not simply a function of experimental error.

The rejection data vs pH for constant salt concentration at fixed pressure is shown in Fig. 2c. The rejection data for the two concentrations studied exhibits a sharp fall in magnitude in the region of pH 3.8 to 4.1. This fall in rejection is attributed to the neutralisation of the membrane at the isoelectric point facilitating free passage of the salt through the membrane by diminishing the effects of charge repulsion and reducing the Donnan potential. The evaluated isoelectric point using this method is in agreement with that obtained from the streaming potential method described previously and subsequent experimentation was conducted in the pH range 3.9 to 4.0 which is representative of the isoelectric point where the membrane charge will be at or very near to neutrality, thus, negating the effects of the membrane charge density on rejection.

4.3. Electrolyte rejection and flux at the membrane isoelectric point

The rejection of various concentrations of salt at the membrane isoelectric point is shown in Fig. 3. Fig. 3a represents the rejection data plotted as a function of applied pressure and clearly indicates that rejection increases as a function of applied pressure and then attains a plateau at high pressure as expected. More interestingly in this case is the fact that the salt rejection actually decreases significantly as the concentration is increased. This behaviour is unexpected and the significant fall in rejection indicates that this is not the result of any amount of experimental error and is a true observation. Fig. 3b illustrates the same experimental data but this time plotted as rejection vs. concentration at different pressures. From this plot, one can clearly observe that the rejection falls as concentration increases. The plot also indicates that there is a pressure dependence to the behaviour in that at high pressures the fall in rejection is linear and at low pressure the fall in rejection is more exponential in nature.

The observed trends demonstrate that there is a phenomenon occurring that is not accounted for in the model described in Section 3. The fact that the rejection is falling with increased concentration would be very typical for a charged membrane and this phenomenon is well documented [48–50]. However, as Eq. (16) would suggest, the rejection for a neutral system should be independent

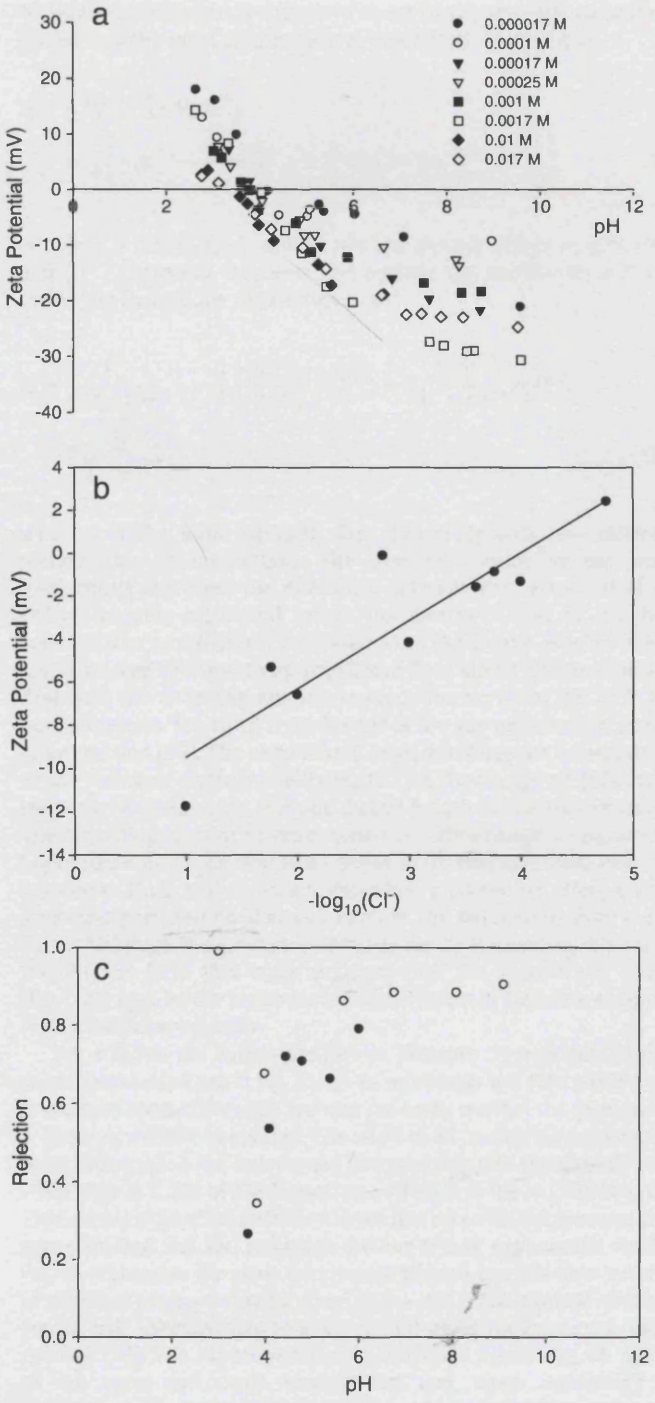

Fig. 2. Experimental results for the Desal DK membrane. a) ζ-potential measurements at various concentrations and pH, b) ζ-potential versus chloride ion concentration at pH 4.0, c) NaCl rejection at 10 bar (○ 0.001 M; ● 0.6 M).

of concentration. So the obvious question to pose is why is this behaviour occurring? One explanation for the behaviour may come from the fact that fixed charge on the membrane is finite in nature, i.e. the charge itself is at a fixed location. A normal assumption when modelling charge is that the charge density is the same in all places and the charge is considered to be a continuum phase. However, in reality the charge is developed from the dissociation of acidic and basic groups as well as from point charges of adsorbed ions. Thus, at the isoelectric point where the global fixed charge is minimal there may be point charges that can be screened

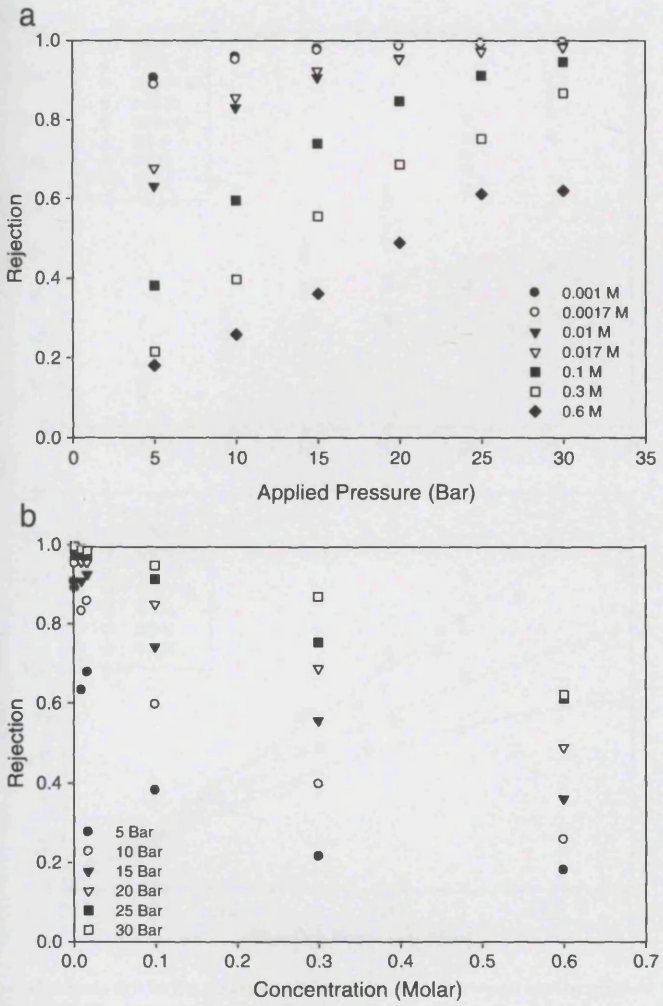

Fig. 3. Rejection for various solutions of NaCl at the membrane isoelectric point. a) Rejection versus applied pressure, b) rejection versus concentration.

by the increase in concentration and that is affecting rejection by allowing the transport of ions. The simplest explanation to consider is that the description of dielectric exclusion is incorrect. The Born model used to describe dielectric exclusion in Eq. (11) is represented as

$$\Delta W_i = \frac{z_i^2 e^2}{8\pi\varepsilon_o a_i}\left(\frac{1}{\varepsilon_p}-\frac{1}{\varepsilon_b}\right) \tag{17}$$

where ΔW_i is the ion solvation energy barrier, z is the ion valence, a_i is the solute hydrodynamic radius (Stokes Radius), e is the elemental electron charge, ε_o is the permittivity of free space, ε_p is the pore dielectric constant and ε_b is the bulk dielectric constant. Simple inspection of Eq. (17) indicates that there is no concentration term present. Indeed, on review of the assumptions contained in the original Born model [51] then the model considers the transport of a single ion from a vacuum into a dielectric media, i.e. there is no implicit account for the fact that multiple ions exist in the system considered here. This being the case, there could well be a justified argument to revisit the original assumptions of the Born model and introduce multiple ions to the derivation.

Other descriptions for dielectric exclusion exist, most notably those considering the phenomenon of image forces. The descriptions of dielectric exclusion as a result of image forces are quite complex in nature and the most simple form is that for slit like [52,53].

$$\begin{aligned}\Delta W &= W\left(0^+\right)-W(0^-)\\ &= r_B\left\{\kappa(0^-)-\frac{\varepsilon_b}{\varepsilon_p}\left[\kappa\left(0^+\right)+\frac{1}{r_p}\ln\left(1-\gamma e^{-2r_p\kappa(0^+)}\right)\right]\right\}\end{aligned} \tag{18}$$

where r_B is the Bjerrum radius, κ is the inverse Debye length, (0^-) and (0^+) represent locations just outside the membrane and just inside the membrane respectively, with

$$\begin{aligned}r_B &= \frac{F^2}{8\pi\varepsilon_b RTN_A}, \gamma = \frac{1-\varepsilon_m/\varepsilon_p}{1+\varepsilon_m/\varepsilon_p}, \kappa(0^-) = F\sqrt{\frac{2I(0^-)}{\varepsilon_b RT}}, \kappa\left(0^+\right)\\ &= F\sqrt{\frac{2I(0^+)}{\varepsilon_b RT}}\end{aligned} \tag{19}$$

where I is the ionic strength. Eq. (18) represents two different mechanisms of interaction. The first two terms in the brace parenthesis represent the difference between the reciprocal of the Debye lengths, calculated inside the nanopore and in the bulk solution. The parameter κ is derived from the Debye–Huckel theory and is related to the activity coefficient for a single charged ion and describes the changing ion–ion interactions between the bulk and pore solutions. The third term describes the ion-polarisation energy of interaction [54]. The exponential term describes the typical decay of any screened electrical field; $2r_p\kappa(0^+)$ is the decay rate [55] and is the ratio between pore size and Debye length inside the nanopore. The screening is most relevant when the dimensionless parameter approaches unity, i.e. for large pores or in this case concentrated solutions. Thus, this equation describes a screening effect on the dielectric partitioning that can explain the behaviour observed in Fig. 3. Although the existence of image forces is questionable for NF membranes [33], this work suggests that the Yaroshchuk model [Eq. (18)] may be the better current description of dielectric exclusion from a fundamental basis.

Fig. 4 shows the membrane flux vs. pressure corresponding to the experiments depicted in Fig. 3. Fig. 4a represents the data plotted as a function of applied pressure and one can easily see that the relationship is linear as would be expected. The effect of increasing concentration is quite pronounced and reduces the observed flux rate significantly from ~160 LMH at 0.001 M (58.5 ppm) to ~73 LMH at 0.6 M (35,000 ppm). This is more than a 50% reduction in the flux rate over the concentration range studied and the reduction follows a near exponential decline. Fig. 4b represents the same information plotted but this time in terms of effective pressure driving force ($\Delta P - \Delta\Pi$). The osmotic pressure for the salt solutions has been calculated using the correction factor method [56]. The experimental data contained within Fig. 4b should all fall upon one single straight line and, when accounting for experimental error, does for the low concentration values. However, the data for the two most concentrated experiments (0.3 M and 0.6 M) does not fall upon this line and noticeably deviates. Taking the final data point for the 0.6 M experiment as an example, the corrected effective pressure using the osmotic correction factor is approximately 25 bar. However, simply interpolating from the rest of the data sets, this value should be closer to 15 bar indicating a 3 fold error. This is most likely due to the fact that the osmotic factors from Ref. [56] are incorrect. In fact, closer inspection of the reference data reveals that the vast majority of the osmotic factors have been determined from pre-war experimentation and may be subject to some error. This suggests that even simple fundamental data sets for physical properties in the concentration ranges of interest for desalination may need to be revisited for accuracy, especially so when considering concentrated brine effluents from the desalination process.

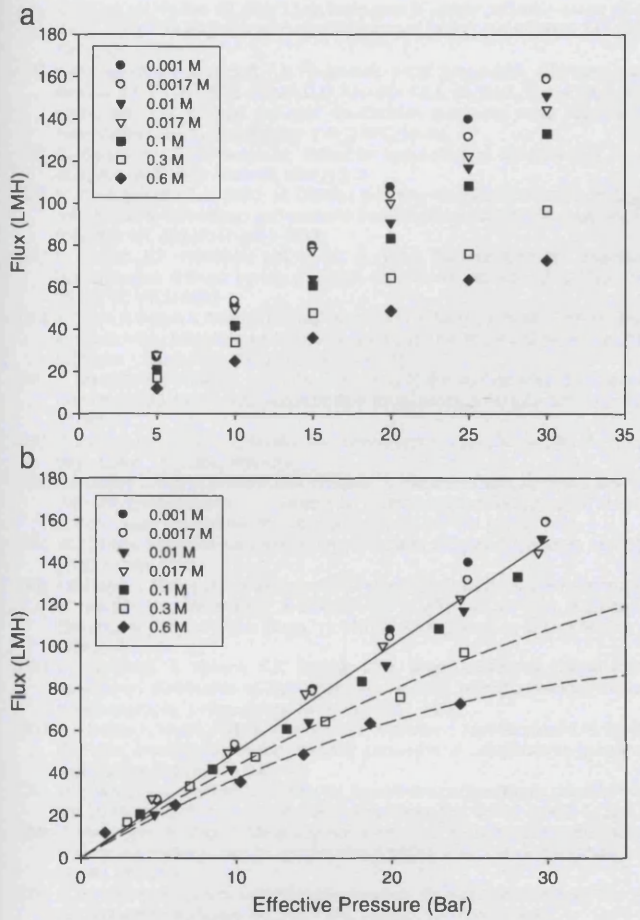

Fig. 4. Membrane flux for the experimental data in Fig. 3. a) Flux versus applied pressure, b) flux versus effective pressure driving force.

5. Conclusions

Nanofiltration plays an important role in sea water desalination, especially pre-treatment operations. Accurate models of nanofiltration are required for the ab initio design, optimisation and scale up of effective industrial processes. The most widely accepted models of nanofiltration are based upon the extended Nernst–Planck equation and have been shown to be highly effective for the modelling of simple salt solutions in the laboratory and have yet to be fully demonstrated for separations of industrial importance. Although there are some examples of multiple ion and solute separations available, these are few and far between and there is a genuine need for further examples when modelling nanofiltration beyond the laboratory into separations of real industrial significance, i.e. multiple ions and solutes at high concentrations.

The current state-of-the-art in nanofiltration modelling has been outlined and the typical solution methodology is explained. The major shortcomings of this theory when applied to sea water desalination have been highlighted and these are mainly related to a lack of understanding of the physical properties and physico-chemical phenomena inside nanopores. Most notably the lack of understanding in relation to activity coefficients, ordering of water layers at the solid–liquid interface and simple physical properties. For example, the study revealed that even simple physical property information such as osmotic pressure for high saline waters may not be completely accurate and needs to be re-evaluated for high salinity applications. Similarly, there needs to be a systematic approach to modelling sea water desalination using the extended Nernst–Planck equation in order to develop accurate short cut methods, like those currently available for other technologies such as distillation, for use by non-specialist scientists and engineers.

Dielectric exclusion from nanofiltration membranes has been further investigated at the membrane isoelectric point. This effectively neutralises the membrane charge and decouples the effects of membrane charge and dielectric exclusion, allowing a systematic independent study of dielectric exclusion only. A rejection study for a range of concentrations representative of sea water desalination was made using sodium chloride as a simple sea water substitute. This study revealed that a significant drop in rejection was observed with increasing concentration. This highlighted the fact that there may well be some screening of the dielectric behaviour at higher concentrations that is not properly accounted for using the Born model. Other models of dielectric exclusion based on image forces were shown to be capable of representing this phenomenon and may be more appropriate for use in modelling from a fundamental stand point.

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